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ABSTRACT

This laboratory manual presents activities for a unit of science within the Biomedical Interdisciplinary Curriculum Project (BICP), a two-year interdisciplinary precollege curriculum aimed at preparing high school students for entry into college and vocational programs leading to a career in the health field. These twenty-five laboratory activities concentrate on biomedical problems in investigating various aspects of the circulatory system, providing concrete experiences for students to explore the heart and blood vessels, blood and its properties, and the urinary tract. (CS)

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SE 031 470

BIOMEDICAL SCIENCE

UNIT III

THE CIRCULATORY SYSTEM IN HEALTH AND SCIENCE

THE HEART AND BLOOD VESSELS;
BLOOD AND ITS PROPERTIES;
THE URINARY TRACT

LABORATORY MANUAL

REVISED VERSION, 1976

THE BIOMEDICAL INTERDISCIPLINARY CURRICULUM PROJECT

SUPPORTED BY THE NATIONAL SCIENCE FOUNDATION

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ACTIVITY 1:

PATHWAY AND FUNCTION OF THE CIRCULATORY SYSTEM

INTRODUCTION:

This activity will acquaint you with the circulatory system. What follows is a programmed text. It contains questions about the heart and blood vessels and clues to help you answer them. By reading it through and answering the questions you will gain a clearer picture of the circulatory system. You will need a sheet of paper or two to write down your answers.

PROCEDURE:

Before reading the first procedure step, take a sheet of paper or a large index card and place it immediately below this line. As you read on, move the sheet down one line at a time until you reach a dashed line. A dashed line means you have come to the end of a question or statement. Stop moving the sheet until you have written out your response. This process of moving the sheet of paper down line by line is to prevent you from accidentally seeing the answer to a question before you have had time to think about it.

QUESTIONS OR STATEMENTS	ANSWERS OR RESPONSES
1. The largest blood vessels in the body are arteries and veins. Arteries carry blood away from the heart. Veins carry blood (back to, away from) the heart.	
2. The smallest arteries are called arterioles. The smallest veins are called venules. Even smaller than these are the capillaries. What are the tiniest blood vessels?	1. back to
3. Venules and veins carry blood (back to, away from) the heart.	2. capillaries
4. Arterioles and arteries carry blood (back to, away from) the heart.	3. back to
5. Blood functions to bring oxygen and other biochemical nutrients to the cells of the body and to remove the wastes made by cells. The nutrients include sugars, amino acids and lipids; and the cell wastes include carbon dioxide and urea. Blood (adds wastes to, removes wastes from) cells.	4. away from

6. Arteries and arterioles carry oxygen-rich blood to all parts of the body. Arterioles branch into capillaries, which bring blood into close contact with the cells. Cells remove some of the oxygen from the blood in capillaries. Venules and veins carry blood from capillaries back to the heart.

The blood in venules and veins should be (high, low) in oxygen.

5. removes wastes from

---

7. This is because the body's cells (add oxygen to, remove oxygen from) blood.

6. low

---

8. On the next page is a diagram of the circulatory system. The person in the diagram faces you. Notice that the person's left arm is on the right side of the page. Right and left are reversed. The outline of several organs is shown by a dashed line around each organ. This diagram is not intended to show an accurate picture. For example, the intestines are really much larger, and the kidneys are really higher up. They are drawn this way for the sake of simplicity.

Which organs in the diagram occur in pairs?

7. remove oxygen from

---

9. Notice all the tubes in the diagram. The white ones represent vessels containing oxygen-rich blood. The black ones represent vessels that contain blood that is low in oxygen. Locate the two vessels labeled (1) in the diagram. Is the blood flowing through these vessels high or low in oxygen?

8. lungs, kidneys

---

10. The vessels labeled (1) are the veins which carry the blood back into the heart from every organ except the lungs. They are the largest veins in the body.

Look at the heart. Note that it has two sides. The right side is shown as black and the left side is shown as white. (Remember that the right side of the person's heart is on the left side of the diagram.)

The right side of the heart contains blood (high, low) in oxygen.

9. low in oxygen

KEY

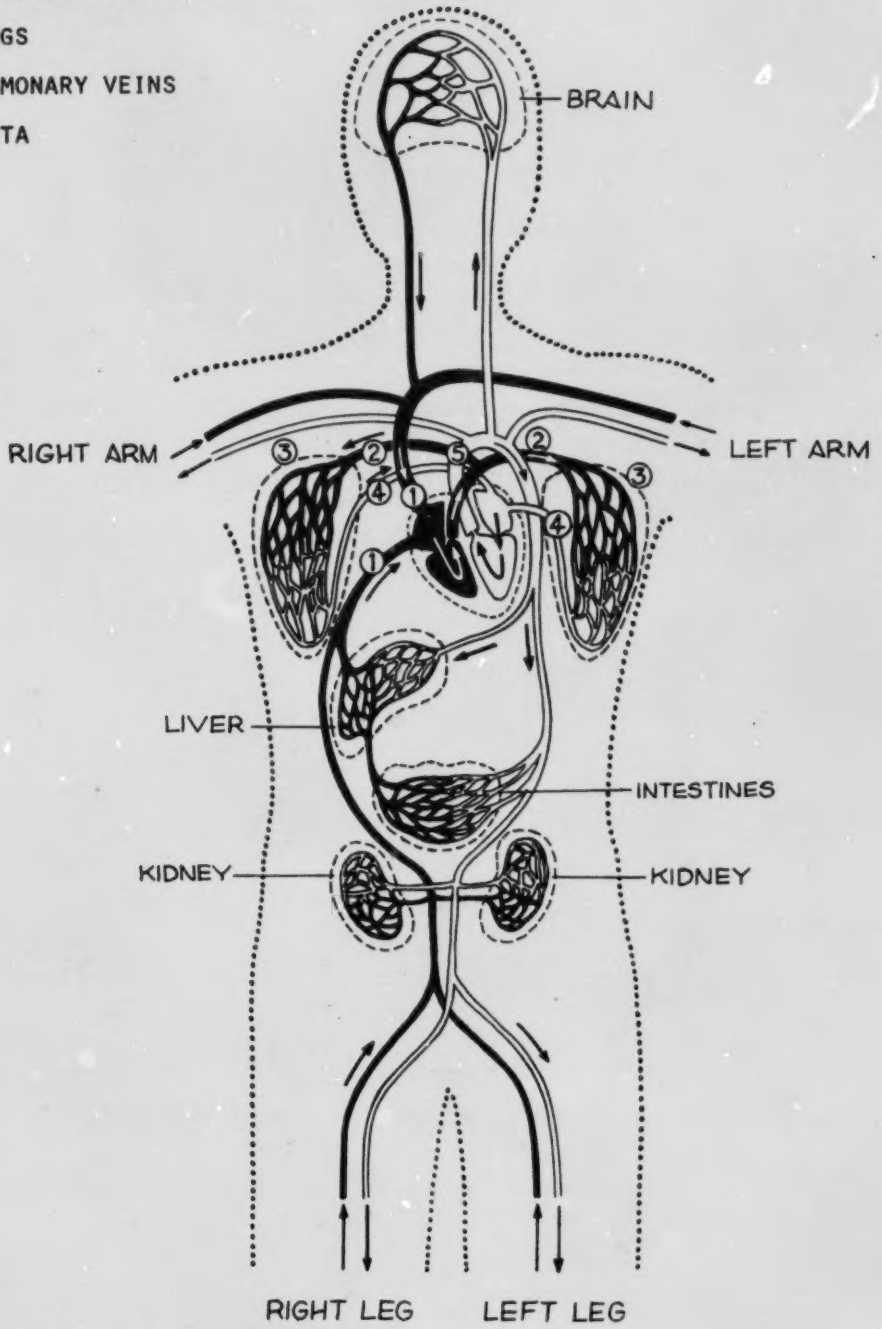
① LARGE VEIN

② PULMONARY ARTERIES

③ LUNGS

④ PULMONARY VEINS

⑤ AORTA



BLACK = OXYGEN-POOR BLOOD
WHITE = OXYGEN-RICH BLOOD

11. Blood from every organ except the lungs ends up in the largest veins in the body. The diagram shows that these veins empty into the (right, left) side of the heart.

10. low in oxygen

12. From the right side of the heart blood is pumped out into the lungs in the vessels labeled (2) (the pulmonary arteries). Is blood that goes to the lungs (3) from the heart high or low in oxygen?

11. right

13. In the lungs blood picks up (oxygen, carbon dioxide).

12. low

14. In addition, blood loses (oxygen, carbon dioxide) in the lungs.

13. oxygen

15. From the lungs, blood goes back to the heart, much richer in oxygen than it was before. It goes back in the vessels labeled (4) (the pulmonary veins). Does the oxygen-rich blood returning to the heart through the pulmonary veins enter the right or left half of the heart?

14. carbon dioxide

16. From the heart, the oxygen-rich blood is now pumped to all the organs in the body <u>except</u> the lungs. The vessels that carry the blood from the heart to the organs of the body are called (arteries, veins).

15. left

17. Note the blood vessel labeled (5) in the diagram. It is called the aorta (pronounced a-OR-tuh). Is this vessel an artery or vein?

16. arteries

18. Blood in an artery cannot go back the same way it came. The blood cannot go directly back to the heart. It has to return to the heart in a different vessel, a vein. Follow the path of the blood from the heart to the intestines via the aorta. To which organ does blood go after it leaves the intestines?

17. artery

19. To which organ does the blood go after it passes through the brain?

18. liver

20. To which organ does the blood go after it passes through the liver?	19. heart
	20. heart

As blood passes through the organs of the body, the cells of the organs exchange materials with the blood. As a result of the exchange, the composition of the blood changes according to the function of the organ. Note the following table for blood exchange by a few selected organs and then continue answering questions based on the table.

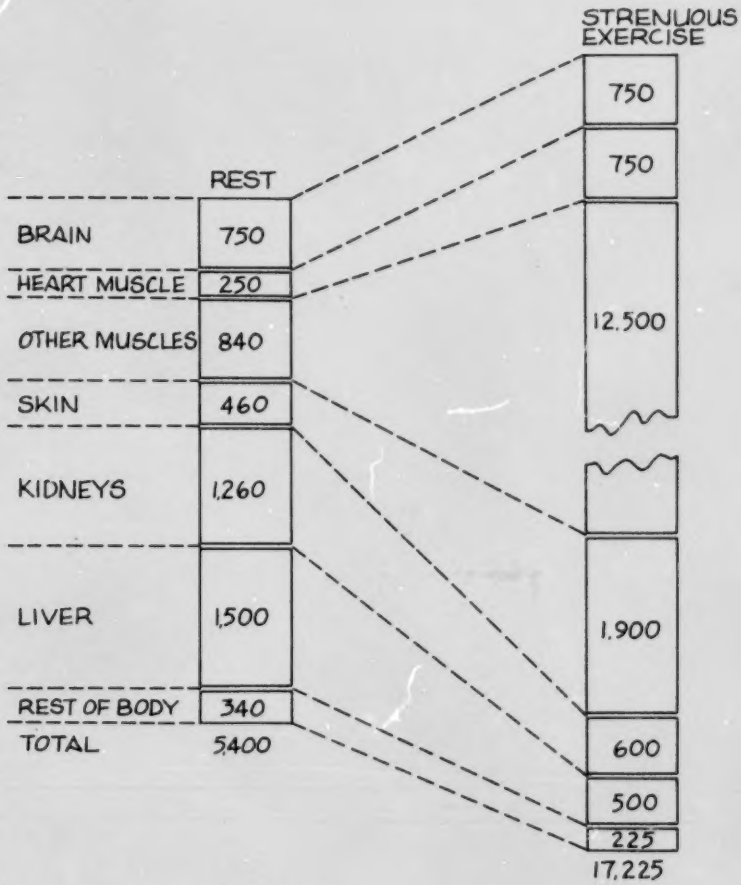
TABLE 1: EXCHANGE OF MATERIALS IN BLOOD IN SELECTED ORGANS

ORGAN	ORGAN REMOVES FROM BLOOD	ORGAN ADDS TO BLOOD
Intestine	oxygen and other nutrients	nutrients including water; carbon dioxide, other cell wastes
Liver	oxygen and other nutrients, worn-out red blood cells, toxins (poisons)	glucose, carbon dioxide, other cell wastes (produced in liver)
Kidney	cell wastes, oxygen and other nutrients	carbon dioxide, other cell wastes (produced in the kidney)
Brain	oxygen and other nutrients	carbon dioxide, other cell wastes
Lungs	carbon dioxide, water, and nutrients	oxygen, cell wastes

21. Which organs add nutrients to the blood?	
22. Which organs remove wastes or toxins from the blood?	21. intestine, liver (glucose), lungs (oxygen)
23. Which organs do <u>not</u> remove oxygen from the blood?	22. liver, kidney, lungs (carbon dioxide)
	23. lungs

Table 2 shows the distribution of blood flow in ml per minute to the various organs of the body.

TABLE 2: BLOOD FLOW TO SELECTED ORGANS DURING REST AND EXERCISE (ml/min)



24. What are the four areas that receive the largest supplies of blood during rest?	
25. Which parts of the body receive more blood flow during exercise than at rest?	24. liver, kidneys, muscles (other than heart), brain
26. Which parts of the body receive less blood flow during exercise than at rest?	25. heart muscle, other muscles, skin
27. Which organ(s) receives the same amount of blood at rest and during exercise?	26. kidneys, liver, "rest of body"
	27. brain

LABORATORY ACTIVITY 2:

OBSERVATION OF CAPILLARY CIRCULATION IN A GOLDFISH

INTRODUCTION:

In this activity you will watch blood flowing through capillaries, arterioles, and venules in a goldfish. The tails of goldfish are nearly transparent, so that the vessels inside are visible. In the human body there is no place where these vessels can be examined as conveniently with a microscope. That's why a goldfish is used.

MATERIALS:

- fish net
- 2 aquaria--one labeled "fresh fish" and one labeled "recovery room"
- live goldfish
- Petri dish (plastic or glass)
- piece of cotton about 8 x 2 cm
- microscope slide
- 12- to 20-cm strip of gauze (3 to 5 cm wide)
- small jar or beaker of aquarium water
- medicine dropper
- microscope
- tape
- lens paper

PROCEDURE:

1. Place a thin, small piece of cotton near one end of the slide. Wet it with aquarium water (Figure 1).

2. Use the net to catch a fish from the "fresh fish" aquarium. Gently place the fish on the glass slide so that the gill region is on the cotton. The gills are narrow slits on each side behind the head. Fold one end of the cotton over the other gill (Figure 2). This will keep the gills wet to enable the fish to obtain oxygen during the activity.

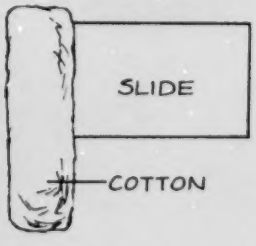
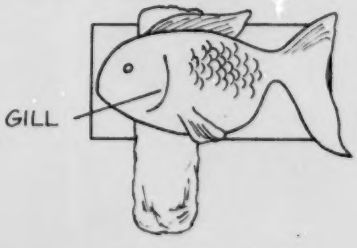


FIGURE 1: Wet cotton on slide.



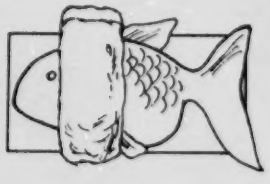


FIGURE 2: Wet cotton wrapped around fish gills.

3. Dampen a gauze strip with aquarium water and firmly, but not tightly, wrap the gauze all the way around the glass slide and the head and body of the goldfish, so that the fish is "bandaged" to the slide with only its tail showing.

4. Set the slide and fish in a Petri dish. Place a small wad of wet cotton on top of where the fish's head and gills are. Use the dropper to add aquarium water to the Petri dish to a depth that just submerges the tail fin. If the water is not deep enough, you may have difficulty seeing the structures through the microscope. Remember that if the gills are not kept wet, the fish will not be able to obtain oxygen. Use the dropper to wet the wad of cotton frequently.

5. Smooth out the tail fin for observation. Be sure to keep the fin submerged in the aquarium water. Add more water with a dropper as needed.

6. Place the Petri dish and goldfish on the stage of the microscope after:

a. removing clips, if any, from the stage;

b. wiping off any excess water from the outer surface of the Petri dish;

c. making sure that the low-power objective is in position and is well above the stage.

7. Tape the Petri dish to the stage, if necessary, to prevent the dish from tilting or falling off.

8. Occasionally an active fish will flip its tail or wiggle out from under the bandage. Return it to the proper position, clean the lens and continue. If the fish is too active, get another fish and remove the first fish to the "recovery room" aquarium.

9. Your observations should be made with the low-power objective (10X) of your microscope. Keep the aquarium water off the objective. If the objective should get wet, clean it thoroughly.

10. Look for streams of blood moving through blood vessels. If you observe a slowing down of circulation in the tail fin, immediately remove the fish to the "recovery room" and get another. (It may be in danger of suffocating.) Notice the various diameters of the blood vessels and the way they branch and rejoin one another. Remember that the view you see through the microscope is reversed. Knowing which way the fish is positioned under the microscope, figure out which moving streams contain blood flowing toward the head and heart. Figure out which direction of flow is away from the head and heart and toward the tail. Now identify an artery or arteriole. Find a vein or venule. How can you tell an arteriole from a venule? (1)

11. Find a capillary. How can a capillary be distinguished from an arteriole or venule? There are two easy observations you can make that will help you distinguish capillaries from other blood vessels.

a. The capillary is so narrow that only one red blood cell can pass through it at a time. Thus you can look for a vessel with red blood cells lined up in single file.

b. Arterioles branch into capillaries and capillaries join into venules. Look for either the branching or joining.

12. Don't forget to keep the fish wet. Add a few drops of water every few minutes.

13. Find an arteriole dividing into capillaries and a venule where capillaries are merging.

14. Note the blood flow in an arteriole. Is it smooth or in spurts? (2) If in spurts, speculate on why it is this way. (3)

15. Note the blood flow in a venule. Is it smooth or in spurts? (4)

16. Find a capillary with flowing blood. Describe the flow. (5)

17. List as many ways as you can think of to distinguish an arteriole from a venule by comparing their appearance under a microscope. (6)

18. Return your fish to water (the "recovery room") as soon as you are finished making your observations.

DISCUSSION QUESTIONS:

1. A fish obtains oxygen through its gills. What carries the oxygen to its tail?

2. How did the diameter of capillaries compare with those of arterioles and venules?

3. During exercise, the arterioles in our muscles become wider. What is the advantage of this? What advantage is there to the fact that the arterioles in our skin become narrower when it is cold?

LABORATORY ACTIVITY 3:

REVIEW OF DIFFUSION AND OSMOSIS

GENERAL INTRODUCTION:

Blood inside capillaries brings nutrients near all the body's cells and carries cell wastes away from cells. The nutrients must move out of the blood to get to the cells, and cell wastes must move from cells into the blood. Materials move in and out of capillaries and cells by the processes of diffusion and osmosis.

In this activity you will observe diffusion and osmosis acting in a plant system and in an animal system.

PART I: CHICKEN-EGG OSMOSIS

INTRODUCTION:

In this part the osmotic properties of a chicken egg will be observed. In the Respiration Unit you saw that osmosis involves the movement of water through a semi-permeable membrane. The membrane allows water to pass through, but certain other substances cannot.

MATERIALS:

- chicken egg
- 10-ml pipet or 50-cm glass tubing
- beaker, 250-ml or larger
- ring stand and clamp
- silicone sealant or candle wax and matches
- pointed instrument (scissors or knife with sharp point)
- mm ruler

PROCEDURE:

1. Hold the egg in the palm of your hand for about a minute, to allow the yolk to settle. This will help prevent the yolk from being broken when the top of the shell is broken. Using the pointed instrument, carefully break a hole in the top of the eggshell which is just large enough for the pipet or glass tubing to fit through. Insert the glass tubing about 2 cm into the egg. There is a membrane beneath the shell. Be sure that the tube penetrates the membrane as well as the shell. Use the silicone sealant or melted wax to seal off the space between the glass and the eggshell to make it watertight.

2. Place the egg in a beaker and support the glass tubing with a ring stand and clamp (Figure 1). Fill the beaker with enough water to cover the egg. Make observations every 10 or 15 minutes, recording the water level in the pipet. If glass tubing is used rather than a pipet, use a mm ruler to measure the water level.

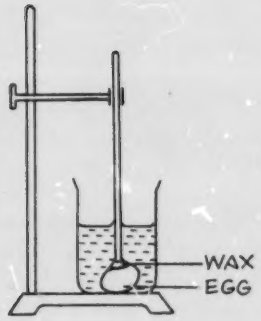


FIGURE 1

PART II: OSMOSIS IN A TOMATO

MATERIALS:

slide	tomato
coverslip	dropper bottle of tap water
microscope	dropper bottle of .85 M NaCl
lens paper	paper towels or other tissues

PROCEDURE:

1. Place a very small piece of tomato on the slide. Use the fleshy part just under the skin, not the skin itself or the seedy part inside. Add 1 or 2 drops of tap water. Place a coverslip on top and gently press the coverslip with a pencil to squash the tomato tissue.

2. Place a drop of NaCl solution on the edge of the coverslip and draw it under the coverslip as you have done before. Remember to place the drop near one edge of the coverslip and touch a piece of paper toweling to the opposite edge of the coverslip as shown in Figure 2.

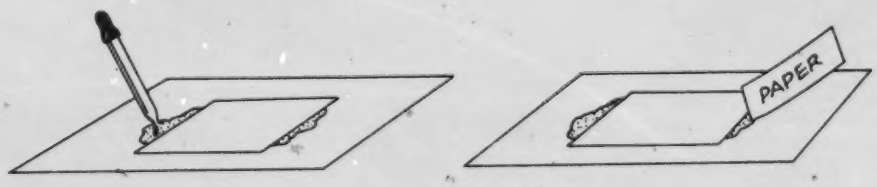


FIGURE 2

3. Observe the cells with a microscope and make a drawing of the cells.

4. Run tap water under the coverslip 4 to 6 times using a dropper and paper, as described in Step 2. Look for a change in the cells. Describe what you see.

DISCUSSION QUESTIONS:

1. What did you observe with the egg set-up?

2. What happens to a tomato cell in tap water?

3. Explain the phenomenon observed in the egg set-up in terms of osmotic pressure.

4. If the protein concentration is less outside a cell than inside, will water diffuse out of the cell? Explain.

5. What is the difference between diffusion and osmosis?

LABORATORY ACTIVITY 4:

INVESTIGATING A FOUR-CHAMBERED HEART

INTRODUCTION:

This laboratory activity will familiarize you with a typical four-chambered heart, much like your own. Knowledge of the basic structure of the heart and the path of blood flow through the heart will help you understand how it performs its normal functions. This knowledge will also help you understand what happens when the heart is diseased. You may find the pictures of the heart in Section 4 of use.

In this activity you will record your observations of the heart and answer some relevant questions. In addition, you will name the parts shown in a numbered diagram of the heart (Figure 2). To prepare for this, you should now number a sheet of paper 1 through 14. When called upon to label a part write the name of the part on your sheet of paper opposite the appropriate number.

MATERIALS:

four-chambered heart
blunt probe
scissors
razor blade or scalpel
dissection pan
model and/or diagrams of the heart (optional)

PROCEDURE:

1. Place the heart in a dissecting tray. Examine the exterior of the heart. To make dissecting more convenient, the heart may have been removed from the fluid-filled sac which surrounds it in the body. The sac is called the "pericardium" (peri = around, cardia = heart).

What is the function of the sac, or pericardium? (1)

2. Find the crown of fat that runs around the heart. This layer of fat marks the boundary between the upper two chambers and the lower two chambers. Feel the heart above and below the fat to see which chambers feel thicker or heavier.

Describe at least two differences between the upper and lower chambers. (2)

The upper chambers are called "atria" (singular = atrium). Each atrium has a small outpocketing called an "auricle." "Auricle" comes from the Latin word for ear. The auricles look a little like ears because of their position on the heart. The lower chambers are called "ventricles." The atria receive blood returning from the body to the heart. The ventricles pump blood out of the heart.

3. Find another deposit of fat that runs diagonally around the lower half of the heart. This deposit marks the boundary of the wall separating the right lower chamber of the heart from the left lower chamber of the heart, or the right ventricle from the left ventricle.

4. Using a scalpel or razor blade, make vertical slits in the walls of both ventricles (see Figure 1 on the following page).

5. Open the two slits and observe the thickness of the walls of each chamber. Describe any differences in the wall thickness of the left and right ventricles. (3)

Use your numbered sheet to label the right and left ventricles and the right and left atria.

6. With a probe, find the wall of muscle inside the heart which separates the ventricles. This is called the "septum" (Latin for "fence"). In some birth defects, there is a hole in the septum, which is usually repaired by surgery.

Label the septum.

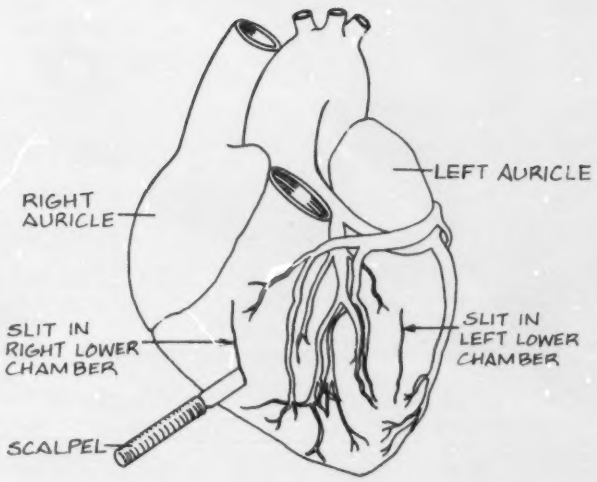


FIGURE 1

7. Introduce the blunt end of the probe into one ventricle and slide it slowly upward along any part of the wall as far as it will go.

The membrane that stops the probe is a heart valve. There is a valve between the ventricle and atrium on either side of the heart. These valves are commonly referred to as "A-V valves" (short for "atrioventricular valves") since they connect the atria and ventricles.

Label the right and left A-V valves.

In which direction does blood flow, from ventricle to atrium or atrium to ventricle? (4)

8. Make the slit in one ventricle longer, extending it up to the atrium to expose the valve. Notice the cords or fibers attached to the valve. Speculate on the function of these cords. (5)

What structure prevents blood from flowing backward, from the ventricle into the atrium? (6)

9. Using the probe, locate the large blood vessels that lead out of the ventricles. The vessel leaving the right ventricle is the pulmonary artery. The vessel leaving the left ventricle is the aorta. (Remember that the left side can be distinguished from the right side by the thickness of the walls of the ventricles.)

Where does the blood in the pulmonary artery go? (7)

Where does blood in the aorta go? (8)

Label the pulmonary artery and aorta.

10. Using scissors, make a lengthwise cut into the aorta. The aorta carries blood from the heart which ultimately goes to every part of the body except the lungs. Extend the cut through the valve and all the way into the ventricle. Examine the valve you have cut through, the aortic valve.

How does the aortic valve differ from the A-V valves in appearance? (9)

What is the function of the aortic valve? (10)

Label the aortic and pulmonary valves.

Which way does blood flow through the aorta: toward the heart or away from it? (11)

11. In the aorta, just above the left ventricle, find the opening of either coronary artery. Follow the coronary artery around the outer surface of a ventricle.

Which organ is supplied with blood by the coronary arteries? (12)

What effect would a blockage of a coronary artery have upon the blood supply to the heart? (13)

12. Carefully insert the probe through the slit in a ventricle, through the A-V valve and into the atrium. Use the probe and your fingers to examine the walls of the atrium.

Compare the thickness of the walls of an atrium to the walls of a ventricle. How are they different? (14)

Speculate on how this difference is related to the different functions of atria and ventricles. (15)

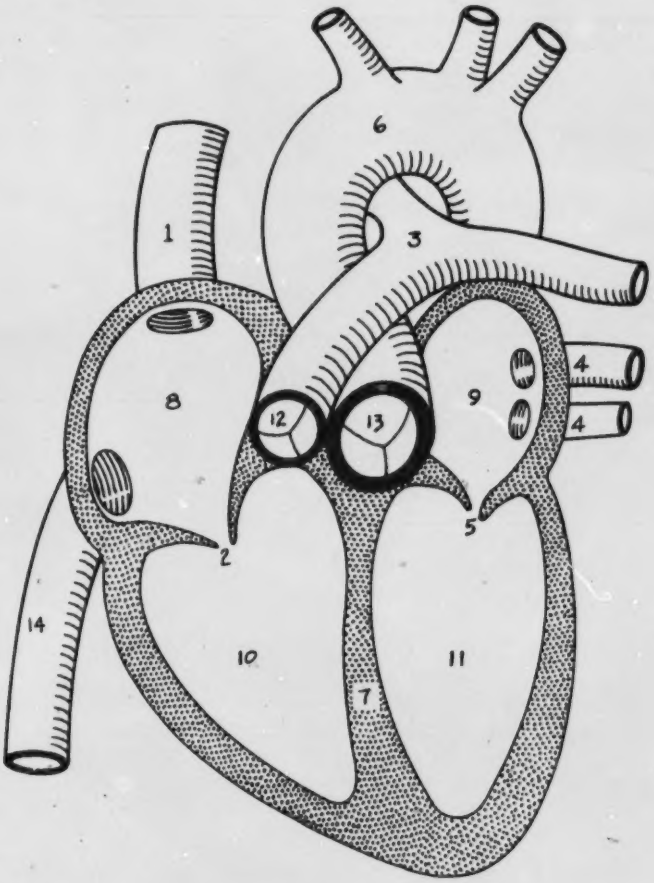


FIGURE 2

13. Locate the blood vessels that enter the atria. The blood vessels entering the right atrium are the upper and lower venae cavae. They carry blood to the heart from the body. The blood vessels entering the left atrium are the pulmonary veins.

The pulmonary veins bring blood to the heart from what organs? (16)

Which vessels, the pulmonary artery or the pulmonary veins, carry oxygen-rich blood? (17)

How are the pulmonary arteries and veins different from the arteries and veins of the rest of the body, in terms of the kind of blood they carry? (18)

14. Compare the structure of the walls of the blood vessels entering the atria with the vessels leaving the ventricles. Notice the thickness and, if the heart is fresh rather than preserved, the elasticity.

How are these vessels different? (19)

Label the venae cavae and the pulmonary veins.

Write, in proper sequence, the pathway of blood flow through the heart and the rest of the body. Name as many structures as you can. Begin with the venae cavae and end with them. (20)

LABORATORY ACTIVITY 5:

HEART SOUNDS, BLOOD PRESSURE AND PULSE BEAT

INTRODUCTION:

In this activity you will become acquainted with some medical instruments used in the diagnosis of heart and vascular disease. Since heart sounds are an important diagnostic tool, you will also study the sounds emitted from normal hearts. In addition, you will take and compare pulse beats under different conditions and at different locations in the body.

The activity will involve five different stations. Note any special instructions given by your instructor. You can start at any one of the stations and, upon completion of that activity, proceed to any one of the remaining stations.

MATERIALS:

STATION	ACTIVITY	MATERIALS
I	Heart sounds	stethoscope cotton and alcohol mm ruler
II	Blood pressure	stethoscope sphygmomanometer cotton and alcohol
III	Pulses, circulation of hand	clock or watch with second hand sheet of graph paper
IV	Arteriolar pulse rate by photocell	glass slide petroleum jelly clock or watch with second hand BIP light source photocell and associated circuitry ring stand, 2 clamps hand lens (optional) small lamp (optional)

(continued)

STATION	ACTIVITY	MATERIALS
V	Ballistocardiography	bathroom scale clock or watch with second hand

STATION I: HEART SOUNDS

INTRODUCTION:

A stethoscope is an instrument for the detection and study of sounds arising within the body. Three common types are illustrated in Figure 1.

FIGURE 1: Three types of stethoscope.

PROCEDURE:

1. As you begin to use the stethoscope, there are a few things you should know in order to use the instrument properly.

a. Clean the earplugs with cotton and alcohol before and after you use the instrument. This will keep them sanitary.

b. While the earplugs are in someone else's ears, do not purposely make sounds which could harm their ears. Noises produced by talking into the stethoscope or knocking or tapping the bell on a solid object can be annoying, since the sounds are loud.

c. While holding the bell in place, hold on to the metal area above the bell. Otherwise, the sounds produced by your fingers rubbing on the bell can mask the heart sounds.

2. So that you get used to the sounds which come through a stethoscope, place the earplugs in your ears and the bell or diaphragm directly on the skin of your forearm about 3 cm below the crease of your elbow. (See Figure 2 on the following page.) While holding the bell in place, keep opening and closing your hands and note the sounds produced. Now move the bell gently around on the skin, and note the sounds. Try the same activities on some part of your body which has clothing covering it. Move the part of the body under the clothing and then move the bell across the clothing. Remember that the sounds you have just heard may not be heart sounds.

3. Now you will locate certain places on the chest which are best for listening to the heart because the sounds carry to them. One good place is right over the lower

tip of the heart. To find it, locate the point where the lowest ribs join at the bottom center of the chest in the front (see point x in Figure 3). Then move to the subject's left 5 to 7 cm. This place is below and slightly to the (subject's) right of the left nipple. It is over the lower tip of the heart (Figure 3).

If you wish, you can feel the heart beating by pressing lightly on this spot.

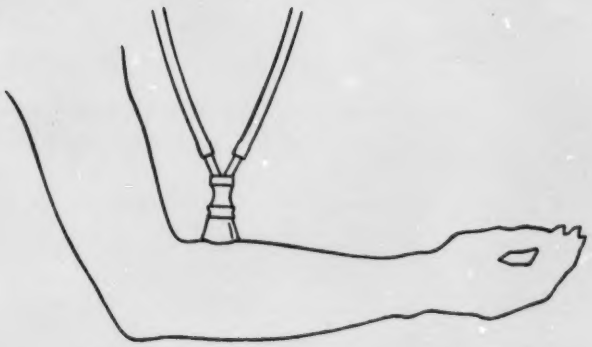


FIGURE 2: Placement of the bell for listening through the stethoscope.

4. Insert the stethoscope earplugs into your ears and place the bell or diaphragm on the subject's chest at the lower tip of the heart. The sound you hear is commonly described with the words "lub, dub." The "lub" sound is the blood slamming against the closed A-V valves. The "dub" sound is the blood hitting the semilunar valves.

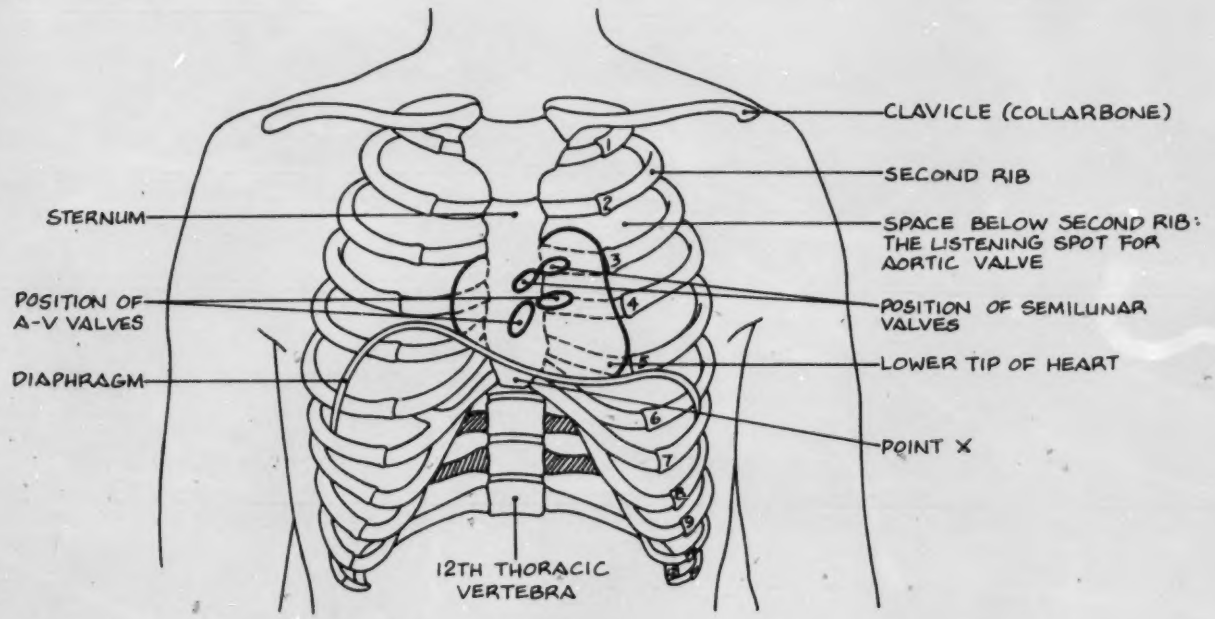


FIGURE 3: How to position the stethoscope close to the tip of the heart.

5. Another good listening spot is where the aortic valve is heard loudest. This is in the space below the second rib and about 2 cm left of the edge of the sternum (Figure 3). The second rib is the rib about 5 to 6 cm below the clavicle (collarbone).

6. Place the bell of the stethoscope on the chest wall at that spot.

In which area, the tip of the heart or the aortic valve listening spot, are the sounds louder? (1)

Compare the sounds near the tip of the heart and the aortic valve listening spot in terms of pitch, duration of sound, and any other differences you may distinguish. (2)

STATION II: BLOOD PRESSURE MEASUREMENT WITH SPHYGMOMANOMETER AND STETHOSCOPE

INTRODUCTION:

Note: Be sure you have read the information on stethoscopes described in the Procedure for Station I, before beginning this activity.

A sphygmomanometer (pronounced SFIG-mo-muh-NAHM-uh-tur) is an instrument used to measure arterial blood pressure. It consists of a cloth cuff, an inflation bulb, an inflatable rubber bag, a pressure gauge, and an indicator for proper placement of the the cuff.

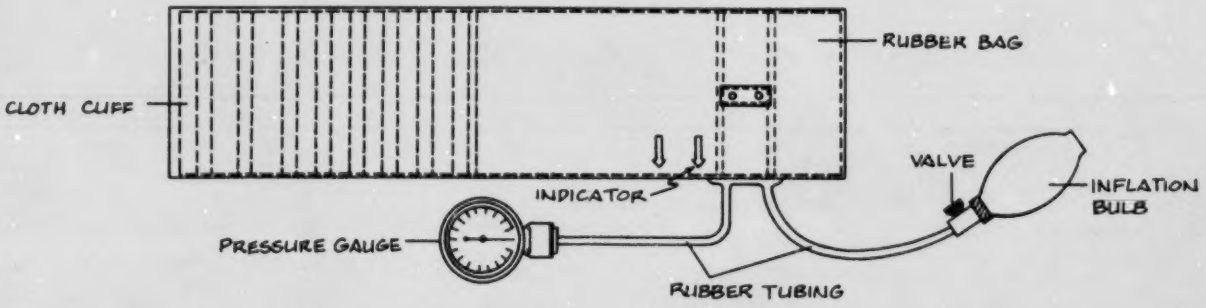


FIGURE 4: A sphygmomanometer.

The cloth cuff contains an inflatable rubber bag. The rubber bag is connected by rubber tubing to the inflation bulb and also to the pressure gauge. This pressure gauge enables one to monitor the pressure within the cuff. The cuff pressure indirectly gives the pressure of the blood in the artery within your upper arm. The pressure is given in units of mm Hg.

When the cuff is applied snugly to the arm, inflation of the rubber bag compresses the tissues under the cuff (Figure 5). At high enough pressures the artery is squeezed so hard that it is forced shut (Figure 6). This happens when the pressure on the outside of the artery is greater than the blood pressure inside. No blood can go through the artery.

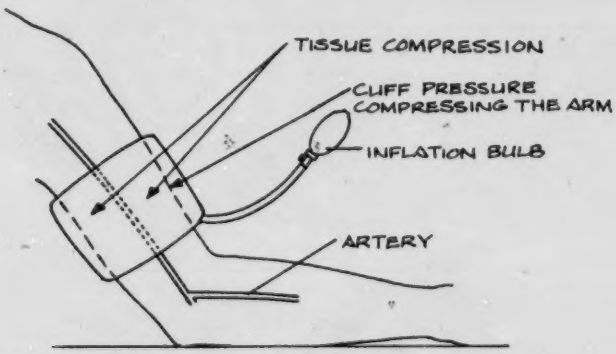


FIGURE 5

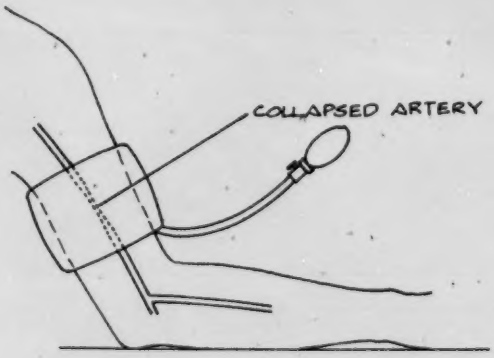


FIGURE 6

Next, the pressure in the cuff is slowly reduced. This eases the pressure on the outside of the artery. When the cuff pressure is reduced enough, a small amount of blood spurts through the artery. This is because the blood pressure at its greatest is slightly larger than the cuff pressure. The blood pressure is large enough to force

open the artery a bit and send some blood through. You can hear the blood going through in spurts with a stethoscope, or you can feel pulses at the wrist. (The sounds are blood rushing through the partially open artery.)

The pressure indicated on the pressure gauge at the time the pulse is first heard or felt indicates the highest pressure of blood in the artery during heart contraction--this is called systolic blood pressure (pronounced sis-TAHL-ik).

As cuff pressure is further reduced, the artery will remain more and more open and the sound will suddenly become softer. At still lower cuff pressures the sound disappears completely, because the artery is once again fully open and blood flow is normal. Blood flowing through fully open arteries makes no sound.

The pressure at which the sounds first disappear is the pressure in the artery during heart relaxation. It is the lowest arterial pressure and is called diastolic blood pressure (pronounced DY-uh-STAHL-ik).

PROCEDURE:

1. The person to be tested should sit down and relax. This is to assure the lowest blood pressure reading. This can be used as a standard if you also take readings following exercise or standing up. Wrap the cuff around the upper arm of the subject so that the bulb and any other tubing is hanging downward (toward the wrist). Fix the cuff snugly, but not too tightly, around the arm. There are several types of fasteners on cuffs. Some have snaps, some have adhesive fabric and some have a long piece of fabric which is wrapped around the arm several times and tucked into itself.

Note the "indicator" often present on the cuff which tells you where to place the cuff. Usually there is an arrow which should be pointing approximately to the middle of the crease in the elbow (Figure 7).

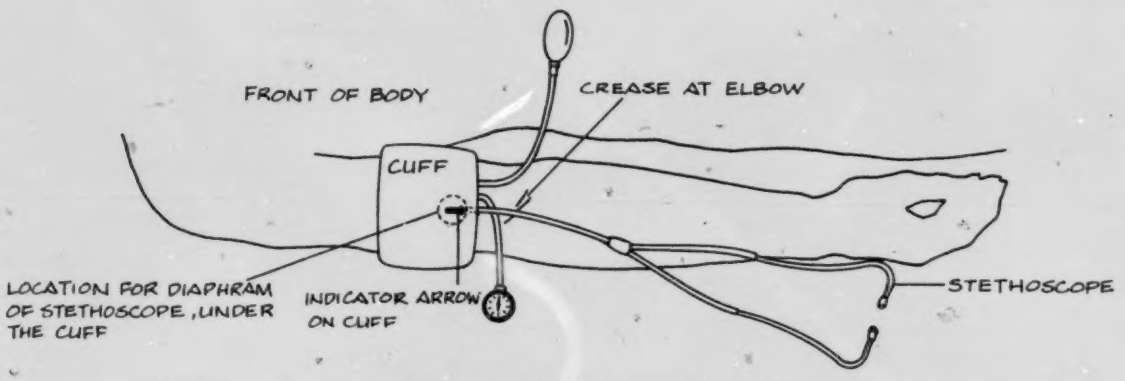


FIGURE 7: Placing the stethoscope for blood pressure.

2. If the stethoscope has a diaphragm, slip the diaphragm under the cuff, leaving it close to the edge (Figure 7). The diaphragm should touch the skin. This can be done with short bells, too. If the stethoscope has a bell too large to stand up under the cuff, hold the bell lightly (not hard) on the skin at the crease of the elbow. You won't be able to hear the pulse until the cuff is inflated.

3. Notice the round knob near the bulb. Turn it clockwise and then squeeze the bulb a few times to inflate the cuff to 150 to 160 mm Hg. At this pressure the sound of pulses in the stethoscope will probably disappear. As you inflate the bag, DO NOT

EXCEED 180 mm Hg on the pressure gauge. At this point, the pressure becomes painful. Also, do not inflate the bag until you are prepared to take a reading.

4. Slowly turn the knob counterclockwise until you reach a point at which the cuff pressure is slowly and steadily decreasing at the rate of 2 to 3 mm Hg per second. Note the pressure when the first sound of pulse appears in the stethoscope, usually between 100 and 140 mm Hg. Record. Repeat the procedure a couple of times. Be sure to give the student a short rest between readings to allow blood to circulate through the arm. It is also important for the subject to remain relaxed.

There are two other ways to observe the systolic pressure. You may wish to compare these to the stethoscope method.

a. Watch the needle jump on the gauge as the air is let out.

b. If you have completed Station III, feel the first pulse at the wrist as the air is let out.

5. To observe the diastolic pressure, listen for the disappearance of the sounds as the cuff pressure continues to decrease below systolic pressure. This is usually between 60 and 90 mm Hg. Record three or more readings of diastolic pressure.

STATION III: PULSE

INTRODUCTION:

At this lab station you will compare the pulse beat at different locations of the body. Every time the heart beats it squeezes a large volume of blood into the arteries. This causes the vessels to expand. After each expansion, the vessels recoil and become narrow again. When you take someone's pulse, what you feel is the expansion and contraction of an artery. The pulse is a result of the difference between the systolic and diastolic blood pressure.

PROCEDURE:

A. The Radial Pulse

1. The radial pulse is the pulse beat in the radial artery. The person being tested should rest his or her forearm and hand on a desk top, palm up. To locate the radial pulse, place your index and middle fingers of one hand on the subject's wrist at a point about 4 cm from the wrist joint on the side of the wrist nearest the thumb (Figure 8). Don't use your thumb, because it contains an artery itself, which may confuse the results.

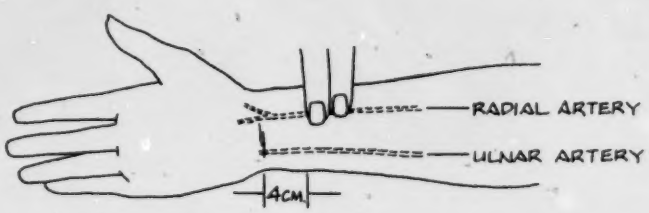


FIGURE 8: Location of radial and ulnar pulses.

2. When you have located the pulse, count the number of beats during a 15-second interval. Record the number of beats, including the uncertainty. Since a 15-second pulse count may be off by one beat in either direction, the uncertainty is ± 1 beat.

3. Repeat Step 2 two more times. Determine the average number of pulse beats during a 15-second interval. Include the uncertainty.

4. Calculate the average number of pulse beats per minute. Multiplying the 15-second pulse count by four will give an uncertainty of ± 4 beats.

5. The ulnar pulse is the pulse in the ulnar artery. To locate the ulnar pulse, place the index and middle fingers of one hand on the subject's wrist at a point about 4 cm from the wrist joint on the side of the wrist nearest the little finger. See Figure 8. This pulse is more difficult to find. Try pressing more firmly. When you have located the pulse, count the number of beats during a 15-second interval and record the uncertainty.

6. Determine the average number of pulse beats per minute for the ulnar pulse.

7. Now you will measure the change in pulse with exercise. Determine the radial pulse for 15 seconds under the following conditions. Record results.

a. While lying down (sitting and relaxed, if not possible to lie down).

b. Immediately after standing up.

c. One minute after standing up.

d. Immediately after strenuous exercise--25 jumping jacks, running in place for 30 seconds, or stepping up and down quickly (using step-test pattern) for 30 seconds. Note the time the subject stops exercising.

e. During the period of recovery from exercise, take the pulse every minute for four minutes after that student has stopped exercising.

8. Use these data to calculate the pulse beats per minute.

9. On a sheet of graph paper, graph the pulse rate against time after stopping exercise.

What happened to the pulse rate immediately upon standing up? One minute after standing up? (3)

Was there a change in the pulse rate during exercise? How much of a change? (4)

What happened to the pulse rate in the minutes after exercise? (5)

What is the advantage to the body of the changes in pulse rate? (6)

B. Blood Flow of the Radial and Ulnar Arteries

INTRODUCTION:

In this activity you should be able to determine the general area of the hand to which the ulnar and radial arteries each carry blood. You and another student will need to work together.

PROCEDURE:

1. Locate both arteries near one of your wrists.

2. With your other hand, press firmly on the radial artery with your fingers and the ulnar artery with your thumb, hard enough to stop the blood flow into the hand.

3. While holding the "experimental" hand up about face high, have someone "milk" the blood from the fingers toward the wrist, until the palm of the experimental hand becomes very pale.

4. Pressing firmly on both arteries, lower the pale hand to about waist level and release only one of the arteries you are closing off. The area in the hand which returns to its normal color is the area "fed" by this one. You may have to repeat the procedure several times. Try releasing the ulnar artery first, and then the radial artery first. Make a drawing of the hand and sketch in the appropriate areas on the drawing. Label one area "supplied by radial artery," and the other "supplied by ulnar artery."

C. Simultaneous Comparison of Pulses

1. Find the carotid pulse, which is the pulse of the carotid artery. Have the subject tilt his or her head slightly backward. Place your index and middle fingers on the subject's throat at a spot that is about 3 cm to the right or left of the larynx and 1 cm above it. (Don't press too hard.)

2. Locate the carotid pulse with one hand and either the radial or ulnar pulse with the other hand.

Do the pulses beat together or does one beat before the other? (7)

Suggest a reason, if they are not simultaneous. (8)

STATION IV: ARTERIOLAR PULSE RATE BY PHOTOCELL

INTRODUCTION:

As blood surges through your body, the amount of blood in any particular place changes slightly with your pulse. This change can be detected in several ways as you can see by performing the activities at this laboratory station. The slight changes in the blood flow through the arterioles in your finger can be seen fairly easily with the naked eye. The pulse can be detected by photocell, too. When you try to shine a light through your finger, most of the light is absorbed by the fingernail, skin, muscle tissues and blood. The change in the amount of blood surging through causes the amount of light shining through your finger to vary. A photocell picks up this varying light and changes it into a varying electrical current which the BIP can detect.

PROCEDURE:

1. Work with another student. The subject should sit or stand with one arm hanging down and the palm facing up. Sunlight or a lamp will make observations easier to see. Put a small blob (about the size of a pea) of petroleum jelly on the end of the subject's longest finger. (See Figure 9 on the following page.) Then press the end of a glass slide gently on the blob of jelly. The region under the slide will become pale. Continue pressing gently until the arteriolar pulse can be seen. Changing the amount of pressure on the fingertip is necessary until just the right amount of pressure is applied to make the pulse visible. You are looking for a pulsating flushing in the pale region of the subject's fingertip. If a hand lens is available, observe the arteriolar pulse with it. Change places with your partner so both of you have an opportunity to view an arteriolar pulse.

2. A lamp and photocell are connected to a BIP. Plug in the BIP.

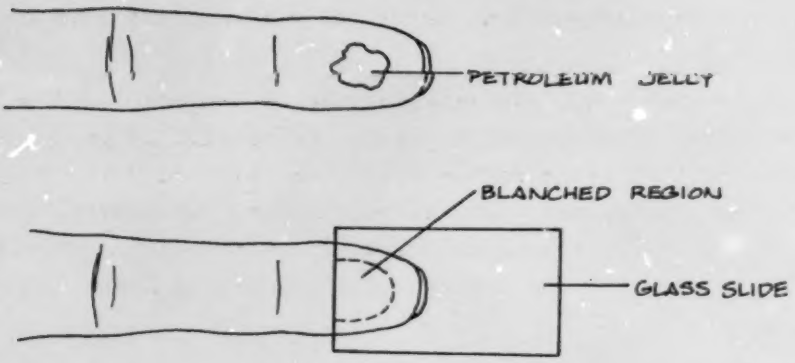


FIGURE 9

3. Mount the photocell and the lamp on the ring stand as shown in Figure 10. It is essential for the light to be directly in line over the photocell. Space them so that when you put your finger between them, it touches both the cell and the lamp at the same time. With your hand palm down, place the end of your finger on the photocell so that it completely covers the cell.

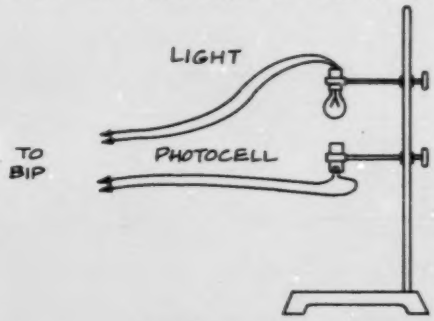


FIGURE 10

4. Use the mA dial to balance the two diode lights on the BIP. The BIP lights permit the change in blood flow to be monitored. It is essential that you not move your finger. Rest your arm on the table or some books so that you can relax your arm. You may have to find just the right pressure with your fingertip. Adjust the pressure by pressing harder or lighter with the finger until it works best. It may take a minute or two.

5. Take your arteriolar pulse by counting the blinking of the BIP diode lights for 15 seconds. Record the number, including the uncertainty.

STATION V: PULSE RATE BY BALLISTOCARDIOGRAPHY

INTRODUCTION:

When the left ventricle of your heart contracts, it moves about 70 ml of blood into the aorta in a fraction of a second. The body reacts (recoils) in response to this sudden spurt of blood by moving slightly.

PROCEDURE:

1. One student crosses one leg over the other and lets it hang down. Relaxing the crossed leg is important. The foot will jump slightly with each heartbeat. The other student counts the pulse for 15 seconds. Record.

2. One student stands on a bathroom scale. If the subject stands perfectly still, the scale reading will change with each heartbeat. Take a few moments to become still. The other student counts the pulse for 15 seconds by watching the scale reading.

3. Note how much the weight reading changes with each heartbeat. For example, it may jump a quarter or a third of a pound. Record this value as closely as possible.

4. The test subject steps off the scale and performs strenuous exercise--25 jumping jacks or running in place for 30 seconds or stepping up and down (using the step-test pattern) quickly for 30 seconds.

Immediately after stopping, the subject steps back on the scale and stands still. The other student notes how far the needle jumps.

Is there a difference in how far the needle jumps before and after exercise? If so, speculate on the reason. (9)

DISCUSSION QUESTIONS:

1. In the blood-pressure test, why does blood flowing through a partially open artery produce a noise, while blood flowing through a fully open artery does not?

2. Compare pulse rates of other members of the class measured before and after exercise. What is the range of values in each case?

3. Compare values for systolic and diastolic blood pressure among members of the class. What is the range of values in each case? What is the normal range for your age group? (See Section 6.)

4. When the pressure on the gauge reads between systolic and diastolic blood pressures, the needle on the sphygmomanometer jumps with each heartbeat. Why?

LABORATORY ACTIVITY 7:

EFFECTS OF TEMPERATURE AND CHEMICALS ON HEART RATE

INTRODUCTION:

The heart is a servant of the rest of the body. It must alter the amount of blood it pumps to meet the constantly changing needs of the body. One way it does this is by altering its heart rate.

The heart rate can be modified by the state of mind (excited, frightened) and by various conditions of the body (exercising, in cold surroundings, feverish, during hypoxia, fainting). The heart rate is controlled by two types of nerves which extend from the brain to the heart.

Body chemicals and drugs called stimulants and depressants also affect the heart rate. Epinephrin (EH-pih-NEF-rin) is an example of a chemical made by the body which causes the heart rate to increase. It is a hormone secreted into the blood. In this activity, you will be able to see the effects of (1) different chemicals and (2) temperature, on the heart rate of a small organism.

The test organism is Daphnia, a tiny aquatic animal related to shrimp, lobsters and crabs. Although this organism is minute, it is a very complex animal and serves as a model for a study of the effects of drugs and temperature on humans.

Daphnia has been selected for use because it is easy to obtain and maintain and, when it is magnified, one can observe its heart at work.

MATERIALS:

culture of live Daphnia
ice water or ice cubes
hot water bath
petroleum jelly

dropper bottles of solutions:
- alcohol
- nicotine
- aspirin

distilled or pond water
clock with second hand
lens paper
depression slide
2 medicine droppers
microscope with light source
toothpick
thermometer
2 beakers, 150- or 250-ml
2 beakers, 50-ml
graduated cylinder, 10-ml
stirring rod
mortar and pestle (or other device for grinding)

PROCEDURE:

1. Take a microscope slide with a well or depression and, with a toothpick, place a tiny dab (size of a pinhead) of petroleum jelly in the center of the cavity.

2. With a medicine dropper, transfer a Daphnia from the culture to the depression in the slide. Absorb excess water with a paper towel or tissue. Work quickly to gently nudge the organism into the dab of petroleum jelly. The Daphnia should stick to the slide but not be totally immersed in the jelly. Try to get the Daphnia on its side so that the heart will be visible when observed in the microscope. Add a few drops of water from the culture. Make sure the organism is always covered with water during the activity.

3. Be sure the bottom of the slide is dry. Place the slide on the stage of the microscope. Adjust the microscope so you can view the organism under the lowest-power lens with a minimum of light. Avoid a bright light because light tends to heat the organism, which affects its heart rate. Experiment with the focus and lighting until you can see internal detail with little light. Turn off the light when not observing the organism.

4. Locate the structures labeled in Figure 1. Note where the heart is located. It is transparent. Often a Daphnia will rhythmically move its legs. Do not mistake the movement of the legs for the beating of the heart. If the legs obscure the heart, try a different organism.

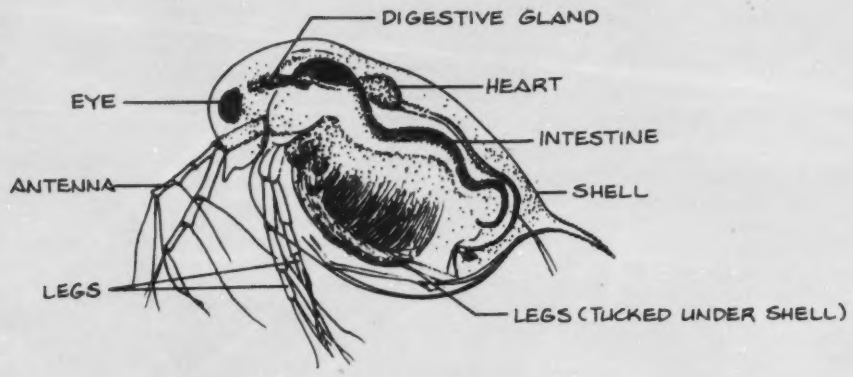


FIGURE 1: Daphnia magnified about 50X.

5. Count the heartbeat for 15 seconds. This operation will be a little difficult since the heart rate is quite rapid. You can count by tapping with a pencil in a line on a piece of paper, as in Figure 2, opposite. Be careful to synchronize what your eye sees with your tapping. Have one of your classmates signal when to begin and when to

stop (i.e., when 15 seconds are over). After stopping, count the number of dots on the paper and record your results, including an uncertainty of ± 1 beat. (See Sample Data Sheet.) Each member of your team should make a count. While other members of the team are counting the heart rate, one person should prepare, in a 150- or 250-ml beaker, a water bath at 5 °C.

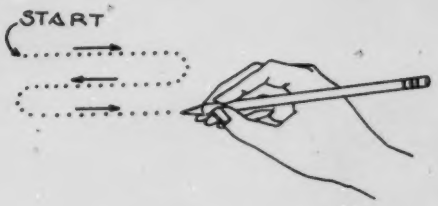


FIGURE 2: Technique for counting Daphnia heart rate.

When finished, average your team's value for the heart rate for 15 seconds and multiply by 4 to obtain beats per minute. Include the uncertainty--multiplying the uncertainty by 4 gives an uncertainty of ± 4 beats per minute. Record.

Keep in mind that the value you have just determined is a "control" heart rate. You will now vary one condition at a time from the control. These heart rates are called "experimental" heart rates as opposed to "control" heart rates. Since only one variable (such as temperature) is changed at a time, any change in heart rate is probably due to that variable.

6. Next you will observe the effects of temperature. Careful work will be needed to obtain meaningful results.

Immerse the slide with the Daphnia in the bath for 2 minutes. This allows the Daphnia to reach the lower temperature.

Remove the slide and dry the bottom. Then make several counts as in Step 5. Since the Daphnia's temperature will begin to rise as soon as it is removed from the cold water bath, work quickly. As the last person begins counting, another person should begin setting up a water bath at a different temperature as described in Step 7.

7. Repeat Step 6 using water baths at 25 °C and 35 °C. Try to use the same Daphnia for all the trials. If the Daphnia dies or is lost from the petroleum jelly, proceed with another.

8. Using the same counting techniques, investigate the effects of the chemicals that are available. Since you will be testing different kinds of chemicals and pills, you will need to keep detailed information on your lab procedures. All solutions should be at room temperature for the chemical tests. Use a different Daphnia with each chemical. Test the solutions provided by your instructor first and then any other materials that are available, as directed in Steps 9 through 12.

9. With a Daphnia specimen on the slide, absorb excess water from the slide with a paper towel or tissue, and use a medicine dropper to add one drop of water from the culture. Use a different dropper to take three drops of clear drug solution (provided by the instructor) and add it to the Daphnia. Determine the heart rate. Record. Wait 1 to 2 minutes and determine it again to allow the drug more time to work. (Be sure to turn the light off during the waiting period so you don't cook the Daphnia or dry up the solution.)

Repeat with any other solutions provided by the instructor. In each case use one drop of water from the culture and three from the drug solution being tested. Rinse the dropper used for drugs between tests.

10. Pills: Record the name of the pill and the list of ingredients from the label. Weigh the pill to ± 0.1 g and record. Grind the pill by wrapping it in a piece of paper and hitting it with a hammer or other hard, heavy object. (The paper prevents the powder from scattering.) If available, a mortar and pestle may be used. (Capsules must first be opened to release their contents.) Then add the powder to 10.0 ± 0.1 ml of distilled or pond water in a 50-ml beaker and stir to break up any lumps. Record the volume of water used (10.0 ml). Let any undissolved particles settle to the bottom.

11. Test your pill(s), using the procedure in Step 9. If the Daphnia's heart stops beating, add 10.0 ± 0.1 ml more of water (distilled or pond) to the drug solution in the beaker to dilute it and repeat the heartbeat count on a new Daphnia. If the heart again stops beating, dilute with another 10.0 ml of water. Be sure to record each dilution in your procedure and all the results on your data sheet. Also record the concentration of each solution in grams of pill per 10 ml water. Clean the dropper before dipping it into different solutions.

12. Other solutions: Record the nature of the solutions and the approximate strength, such as "coffee, undiluted" or "cola, 1:1 dilution."

DISCUSSION QUESTIONS:

1. Compare the number of heartbeats per minute you recorded for each drug to that of your control. Use your comparison to classify the drugs as either stimulants or depressants of heart rate.

2. How does your classification of the drugs compare to that of other members of the class? Suggest reasons for any differences.

3. What are some of the possible sources of error in interpreting the effects of the drugs and chemicals?

4. Why was it important to use a different Daphnia for each chemical test?

5. Compare the heart rates you recorded for each temperature bath. What general effect does temperature appear to have upon heart rate?

6. Speculate on the extent to which the results obtained can be generalized to human beings.

SAMPLE DATA SHEET: EFFECTS OF TEMPERATURE ON DAPHNIA HEART RATE

Temperature	Counter's Name	Number of Heartbeats per 15 sec	Average Number of Heartbeats per minute
Room Temperature (Step 5)		±	
		±	
		±	
		±	
	Average no. heartbeats per 15 sec	±	±
5 °C		±	
		±	
		±	
	Average no. heartbeats per 15 sec	±	±

LAPORATORY ACTIVITY 8:

ELECTROCARDIOGRAPHY

INTRODUCTION:

As the heart muscle beats, a current of electricity passes through the heart muscle and creates a characteristic electrical pattern. This electrical pattern can be recorded by connecting a person to a device which receives the electrical impulses from the person's heart, amplifies them, and produces a tracing on tape (Figure 1). This tracing is called an electrocardiogram (ECG).

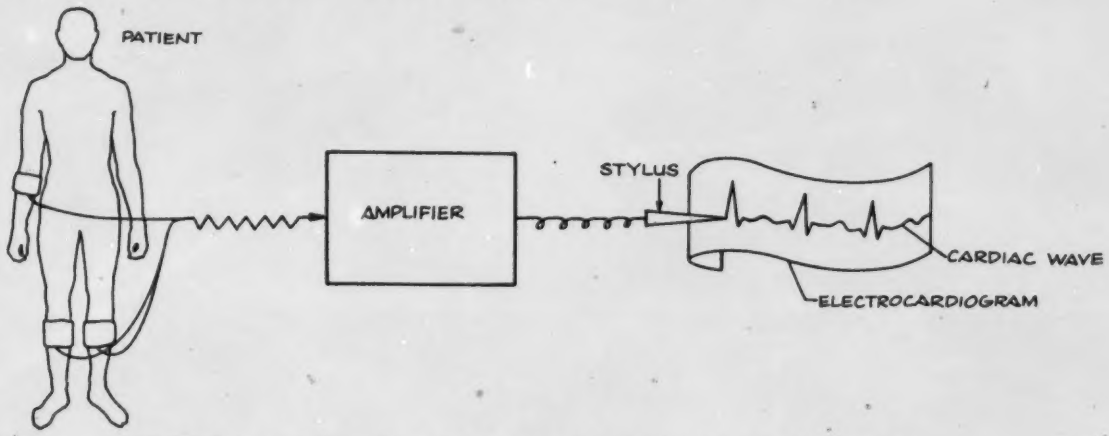


FIGURE 1: Schematic diagram of an electrocardiograph.

In this activity you will program the BIP to function as an electrocardiograph and observe your heart's electrical pattern.

MATERIALS:

- BIP
- electrode with unshielded cable (thin wire)
- 2 electrodes with shielded cable (thick wires with plugs)
- 18-KΩ resistor with two leads
- 3 elastic bandages or strips of 2" gauze, each 3 ft long
- roll of masking tape or fasteners for elastic bandages
- acetone
- cotton or cotton balls
- electrode paste

PROCEDURE:

1. Program the BIP as shown in Figure 2. (Additional connections are shown for use of an oscilloscope, in case one is available.)

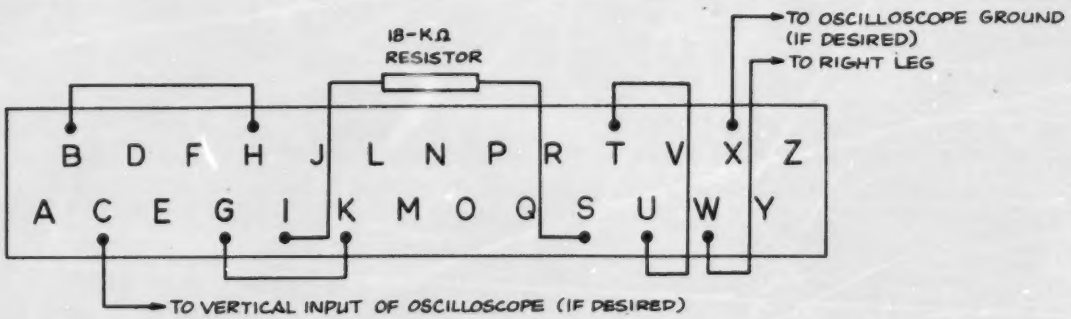

18-KΩ RESISTOR
TO OSCILLOSCOPE GROUND (IF DESIRED)
TO RIGHT LEG
B D F H J L N P R T V X Z
A C E G I K M O Q S U W Y
TO VERTICAL INPUT OF OSCILLOSCOPE (IF DESIRED)

FIGURE 2: Programming the BIP for electrocardiography.

2. Connect a student to the BIP. (Don't plug in the BIP until Step 9.) First, have the student sit on a chair. Then, using acetone (or soap and water), thoroughly clean an area on the parts of the body numbered 1, 2 and 3 in Figure 3. The cleaned areas should be somewhat larger than the size of the ECG electrodes. This is done to remove skin oils which could prevent good electrical contact. This is very important. Poor electrical contact between the electrodes and the body is one of the chief causes of failure of this activity.

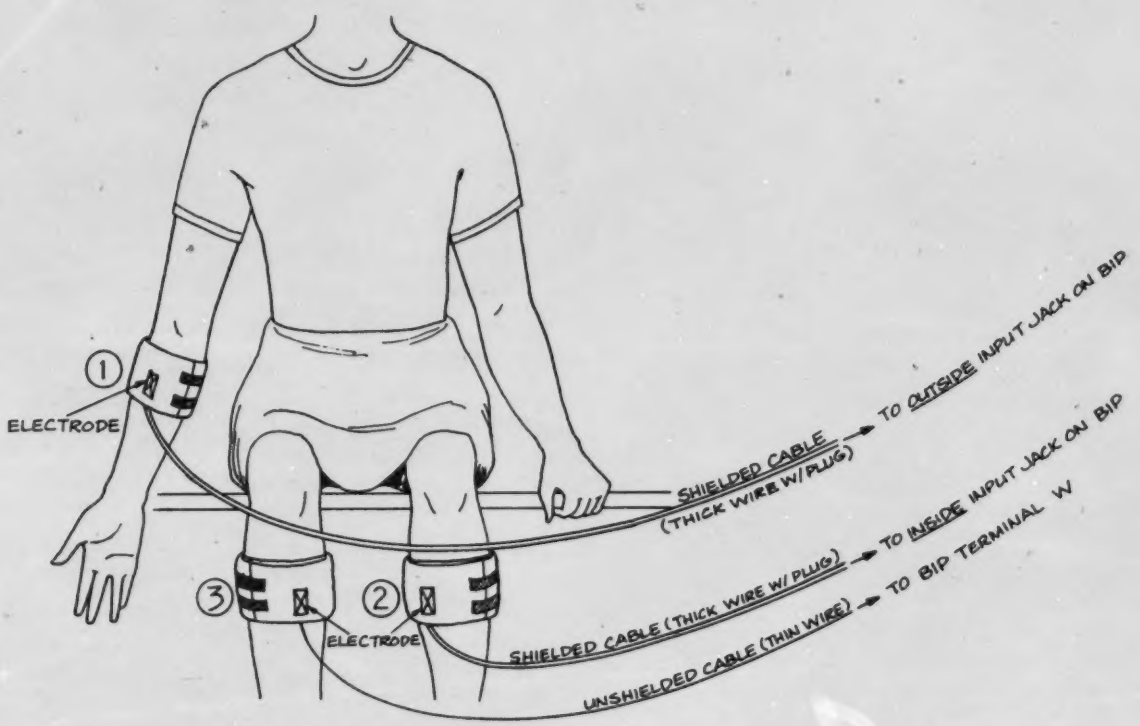


FIGURE 3: Electrocardiograph connections.

3. Smear electrode paste on one of the electrodes attached to a shielded cable. Bandage this electrode to body site #2 in the diagram. Be sure the electrode does not slip from the cleaned area during bandaging. Smear electrode paste on the other electrode attached to a shielded cable and bandage it to body site #1.

4. Repeat this procedure for the remaining electrode. (This electrode should have an unshielded cable.) Bandage the electrode to body site #3.

5. Observe the BIP and note the two input jacks in the bottom right hand corner of the front panel (labeled #8 on Figure 4). Insert the electrode lead from the left leg into the inside input jack and the electrode lead from the right arm into the outside input jack (Figure 4). Be sure not to attach any of the electrode leads to the pH input jack (Jack #1 in Figure 4, opposite).

6. Insert the electrode lead from the right leg (the one with the unshielded cable) into the W terminal on the BIP.

7. Turn the milliamp (mA) dial to 100 (#4, Figure 4).

8. Set the slotted gain control about midway in its range (#7, Figure 4).

9. Check all of your connections to be sure they are correct. Then plug in the BIP.

10. One diode light should be on continuously. We will call this the "negative" diode. The other diode is the "positive" diode. It should be off continuously. If it is not, the gain control setting is too high. Turn the slotted control (#7, Figure 4) slowly in a counterclockwise direction until the positive diode remains off.

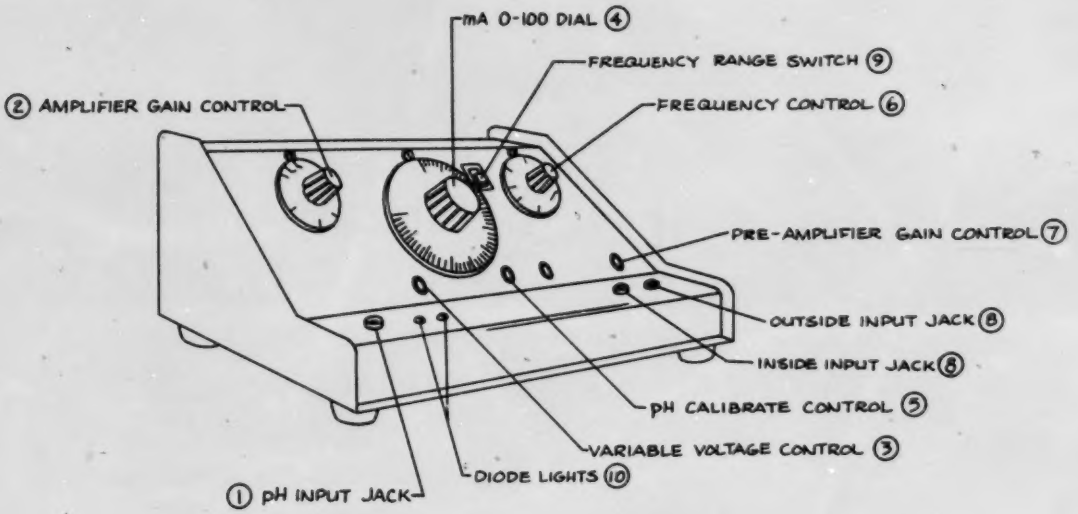


FIGURE 4: Front view of the BIP.

11. Slowly decrease the mA setting until the positive diode just flicks on and off (while the mA dial is held steady). Figures 5A and 5B show what has been happening. Reducing the mA reading is equivalent to lowering the height of the dashed line in the diagrams. The positive diode is on only when the cardiac wave form is above the dashed line. In Step 10 all of the wave form was below the mA setting, and the positive diode was off at all times. Figure 5B shows that, at the lower mA setting, the positive diode will come on briefly once each heartbeat, when the peak of the R wave occurs.

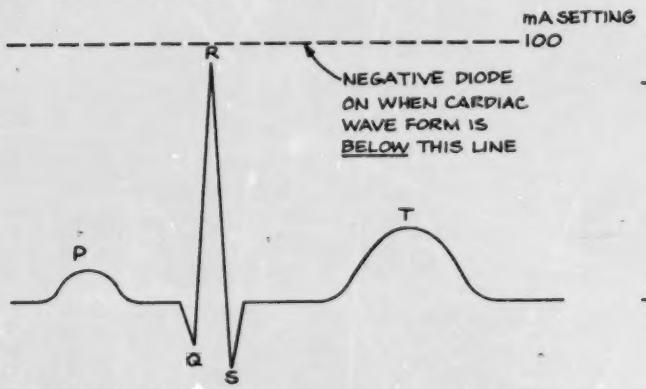


FIGURE 5A: Negative diode on continuously with mA setting of 100.

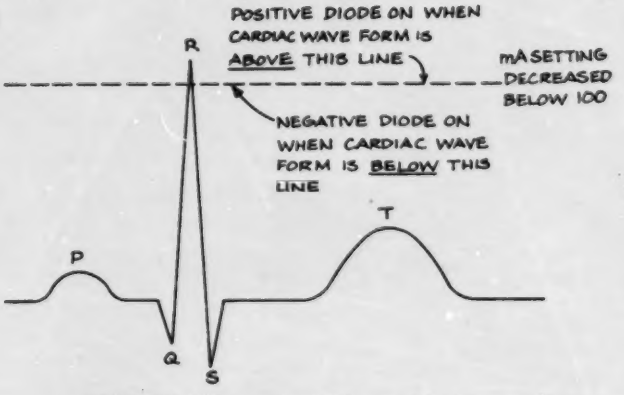

POSITIVE DIODE ON WHEN CARDIAC WAVE FORM IS ABOVE THIS LINE
R
mA SETTING DECREASED BELOW 100
NEGATIVE DIODE ON WHEN CARDIAC WAVE FORM IS BELOW THIS LINE
T
P
Q
S

FIGURE 5B: Positive diode on for each peak of R wave.

12. Figure 6 shows the effect of further decreases in the mA setting, as called for in the remainder of the procedure.

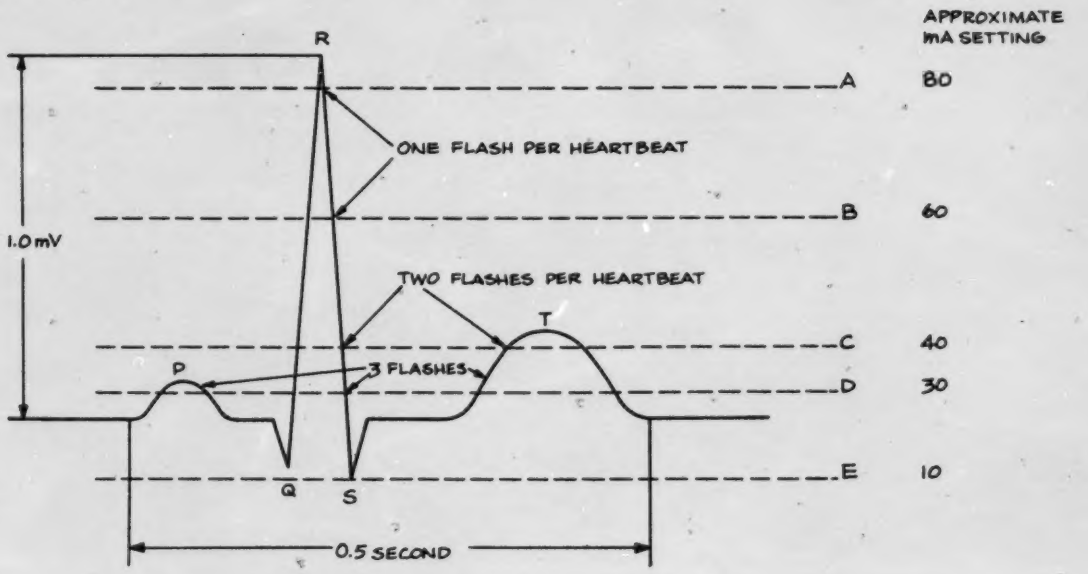


FIGURE 6: The relationship between mA setting and the ECG wave form.

Referring to Figure 6, when the mA dial is at "A" setting (as in Step 11) the positive diode will flash very briefly each time the R wave occurs. At setting "B" the flash will be longer because the R wave is wider. At setting "C" the R flash will be still longer and a second flash will also occur due to the T wave (Step 13). At setting D, a third flash, due to the P wave, will be seen (Step 14), followed by the R wave flash, and finally by a still longer T wave flash. The setting at D is tricky because if the dial is moved further toward setting "E" the diodes will begin to display signals having to do with the Q and S waves. The mA settings shown in Figure 6 are very approximate. Do not be concerned if your settings differ from the ones shown.

If you have difficulty in Steps 13 or 14, the gain control setting may be too low. If necessary, try turning the slotted control slightly in a clockwise direction.

13. Once again slowly decrease the mA setting until the positive diode flicks on and off twice during the same heartbeat. The positive diode is now displaying the peak of the T wave, as well as the R wave (Line C in Figure 6).

14. Again slowly decrease the mA setting until the positive diode flicks on and off three times in one heartbeat. The positive diode is now displaying the P wave peak, as well as the R and T waves (Line D in Figure 6). To make it easier to see the flashes of the positive diode, you might wish to cover the negative diode with masking tape or work in a darkened room.

DISCUSSION QUESTIONS:

1. Draw a typical electrocardiogram pattern and label the P, QRS, and T wave forms.

2. Match the wave with one or more cardiac-cycle events.

P	A. prepares ventricles for next activation wave
QRS	B. immediately precedes contraction of the atria
T	C. immediately precedes contraction of the ventricles

3. Distinguish between an electrocardiogram and an electrocardiograph.

ACTIVITY 9:

ESTIMATING RISK OF CORONARY HEART DISEASE (CHD)

INTRODUCTION:

In this activity you will review some of the case histories in the Nutrition and Respiration units. From these case histories you will try to find clues to whether the individuals described are at high or low risk of coronary heart disease (CHD). For those individuals at high risk, you will be asked to suggest how they could modify their lifestyles to lower their risk of CHD.

We have periodically spoken of factors thought to be related to the occurrence of heart disease. For example, if all other factors are equal, a person with a poor diet with respect to CHD, (i.e., too much saturated fatty acids, too much sucrose) is thought to be more likely to end up with CHD than a person on a good diet (i.e., low in saturated fatty acids and sucrose). This does not say that either individual necessarily will or won't have CHD--we are dealing with statistical relationships rather than with a simple cause-and-effect relationship. In general, the higher your total risk factor, the more likely that you will get CHD. In other words, the relative frequency of CHD will tend to be higher in a group of people with high risk factors. It is important to learn about the CHD risk factors to avoid loading the dice in favor of CHD. Avoiding unnecessary risks is an important part of preventive medicine.

For our study of risk factors, we will use Table 1, on the following page. This table is modified from one produced by the Michigan Heart Association. Similar tables of CHD risk factors have been produced by different groups. Some charts include a few different risk factors such as personal stress, vital capacity and diabetes. In addition, charts of heart-risk factors are not identical in the way they rate the relative importance of each factor. Some rate nutritional factors highest, others rate smoking or blood pressure highest. But all of these charts are useful. They tell people whether they should be concerned about the risk of CHD, and they suggest ways for individuals to lower their risk.

In Table 1, nine risk factors are listed. For each risk factor, the degree of risk increases from left to right. Note that for Age 10-20, the risk is 1, but for Age 61 and over, the risk factor is 8. This means that people over 60 are considerably more likely to have CHD than those under 20. (It does not necessarily mean that the older individuals are eight times as likely to have CHD than the younger group.)

TABLE 1: SCORING HEART-RISK FACTORS

1. Age	10 to 20 1	21 to 30 2	31 to 40 3	41 to 50 4	51 to 60 6	61 and over 8
2. Sex	Female under 40 1	Female 40-50 2	Female over 50 3	Male 5		
3. Weight	More than 2 kg below standard weight* 0	Standard weight ± 2 kg 1	3 to 10 kg overweight 2	11 to 30 kg overweight 3	31 to 40 kg overweight 5	40 or more kg overweight 7
4. Blood cholesterol level (mg per 100 ml plasma)	≤ 180 0	181-205 1	206-230 2	231-255 3	256-280 4	281-300 5
5. % of total calories from saturated fatty acids	≤ 10 0	11-20 1	21-30 2	31-40 3	41-50 4	> 50 5
6. Systolic blood pressure	100-120 1	121-140 2	141-160 3	161-180 4	181-200 6	> 200 8
7. Tobacco smoking	nonsmoker 0	cigar and/or pipe 1	≤ 10 cigarettes a day 2	11-20 cigarettes a day 4	21-30 cigarettes a day 6	> 30 cigarettes a day 10
8. Exercise	intense at work and recreation 1	moderate at work and recreation 2	desk or office work and intensive recreational exertion 3	desk or office work and moderate recreational exertion 5	desk or office work and light recreational exertion 6	complete lack of exercise 8
9. Family history**	no known family history of CHD 1	1 relative had CHD after age 60 2	2 relatives had CHD after age 60 3	1 relative had CHD before age 60 4	2 relatives had CHD before age 60 6	3 relatives had CHD before age 60 7

*"Standard weight" is the normal weight based on tables.

**Only "close" relatives should be included--parents, grandparents, brothers and sisters. There may be a combination of close relatives with CHD, but with some under 60 when they first showed signs of CHD and others over 60, it is tricky to estimate the factor. For example, suppose a person has two relatives with CHD, one under 60 and one over 60. The risk must be greater than 4 and less than 6, so it should be about 5.

Age is only one of several factors that influence the likelihood of a heart attack. It is the total of all the risk factors that provides useful information. Let's consider an example to illustrate this point.

Suppose we wished to determine the risk of CHD for Rose, a 29-year-old woman. Assume that Rose is one kg above her standard weight, that her blood cholesterol level is 200, her systolic blood pressure is 120, she smokes a pipe (no cigarettes), works outdoors in a construction crew and is an enthusiastic swimmer (practices almost daily and competes in meets). Also assume that 30% of the calories consumed by Rose are from saturated fatty acids. Finally, assume that both of her parents and her grandfather had CHD in their fifties. How great is Rose's risk with regard to CHD? To answer this question, we must score Rose on each of the nine risk factors and total the score (Table 2).

TABLE 2: TALLYING ROSE'S SCORE

RISK FACTOR	ROSE'S SCORE
Age	2
Sex	1
Weight	1
Blood Cholesterol Level	1
% of Total Calories from Saturated Fatty Acids	2
Systolic Blood Pressure	1
Tobacco Smoking	1
Exercise	1
Family History	7
Total Risk	17

From Table 2, we see that Rose's total risk of CHD is scored as 17. Since we are not told very much about Rose, the correctness of the scoring could be questioned. For example, we are not told what Rose does on the construction crew. You might argue that her score for exercise could be "3" rather than "1." However, the total risk should still be close to 17. What does a total risk of 17 mean? Is it good or bad? To answer these questions, refer to Table 3 on the following page.

According to Table 3, Rose's risk of CHD is below average. Keep in mind that this is a statistical evaluation--it is not a diagnosis. But it does tell Rose that she is at reasonably low risk at the present time. Of course, Rose should keep in mind that as she ages, her risk of CHD will increase. If all of her risk factors except age remain the same, when she is 62 her total risk will have increased to 25. (Note that the "sex" category is also affected by age.)

Recall that Rose has three relatives who had CHD before age 60. If you considered only this factor, you might expect that she would be at high risk of CHD. But the true risk is a function of many factors. A person can have a high score

for one or two risk factors and yet not be a likely candidate for CHD. Note also that many of the risk factors measure things that can be changed by an individual. In other words, people determine their own risk of CHD to a considerable extent.

TABLE 3: EVALUATING TOTAL RISK FACTOR

SCORE	RISK
5-11	well below average
12-18	below average
19-24	average
25-31	moderate
32-40	high
41-63	much too high

In this activity, you are to review four case studies. Reread each case study. For each, figure out the risk of CHD based on Tables 1 and 3. Since the case studies do not give all the information you need to determine total risk, additional information is given below.

CASE 1: Tom Young (Unit I, Section 41)

(Assumptions to make)

20 pounds overweight (0.45 kg = 1 lb)
blood cholesterol level: 235
% of total calories from saturated fatty acids: 44
blood pressure: 160/90
smoking (cigarettes): 2 packs/day
exercise: see Section 42 and p. 261 (Unit I)
family history: one grandfather had early CHD

CASE 2: Fred Stone (Unit II, Section 2)

blood cholesterol level: 260
% of total calories from saturated fatty acids: 44
blood pressure: 170/100
smoking (cigarettes): $1\frac{1}{2}$ packs/day
works as a clerk
most strenuous exercise: watching football
family history: father (see case history in Section 2); older brother and grandfather had CHD in their 60's

CASE 3: Maria (Unit II, Section 6)

weight: 5 lb underweight
blood cholesterol level: 185
% of total calories from saturated fatty acids: 24
smoking (cigarettes): 3 packs/day
exercise: plays ping pong at home and takes occasional walks on the beach
family history: no cases CHD

CASE 4: Mrs. Jones (Unit II, Section 33)

blood cholesterol level: 270

% of total calories from saturated fatty acids: 41

blood pressure: 160/100

non-smoker

family history: father died of a heart attack in his 50's

DISCUSSION QUESTIONS:

1. Which of the individuals has the highest risk of CHD? Which has the lowest risk of CHD?

2. For each individual at high risk, what specific advice would you give them to decrease their risk of CHD?

3. If a person has a total risk of 35, will they necessarily eventually have CHD? Explain.

4. If a person has a total risk of 10, can they be sure that they will not become a victim of CHD?

LABORATORY ACTIVITY 10:

MEASURING THE HEMOGLOBIN CONCENTRATION OF BLOOD

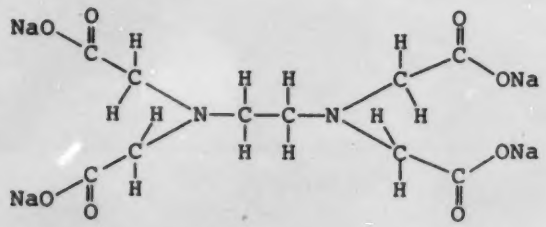
INTRODUCTION:

In Unit I on Respiration you learned that almost all of the oxygen carried in the blood is bound to the hemoglobin (abbreviated Hb) molecule. Since Hb carries O_2, the amount of oxygen the blood can transport depends on the amount of hemoglobin present.

A decrease in hemoglobin concentration, and the consequent reduction in oxygen-carrying capacity of the blood results in the condition known as anemia. A person is considered to be anemic if the blood hemoglobin concentration falls below 12 g Hb/100 ml blood for women and 14 g Hb/100 ml blood for men.

Several techniques are available to the medical lab technician for determining the hemoglobin concentration of a blood sample. One of the more accurate methods is the oxyhemoglobin method which you will be using in this activity. (Oxyhemoglobin is the name given to hemoglobin when it has oxygen atoms attached to it.) As blood is exposed to air, the hemoglobin molecules combine with O_2 to form oxyhemoglobin which is red. The concentration of oxyhemoglobin is then determined by colorimetry.

The directions call for mixing the blood with a solution of EDTA-Na_4 (ethylenediaminetetraacetic acid, tetrasodium salt). This salt (see formula on following page) has the ability to bind tightly to metal ions. It has two functions in the procedure: (1) to tie up metal ions that may be present in tap water (metal ions interfere with the analysis), and (2) to provide an alkaline environment which ensures that the hemoglobin is oxidized as fully as possible.



Tetrasodium salt of ethylenediaminetetraacetic acid ($EDTA\text{-}Na_4$)

MATERIALS:

tetrasodium salt of ethylenediamine-tetraacetic acid solution ($EDTA\text{-}Na_4$)

sterile blood lancet

alcohol cotton wipe packet

micropipet, 20-µl

pipet, 10-ml

2 cuvets, 16 x 125 mm, Pyrex

BIP colorimeter

glass-marking pencil

cotton or tissues

PROCEDURE:

1. Program the BIP for colorimetry and plug it in. (It should be allowed to warm up for at least 10 minutes before use.)

2. Label one cuvet "A" and the other "B".

3. Pipet 5 ± 0.1 ml of $EDTA\text{-}Na_4$ solution into each cuvet.

4. Puncture your finger as you did in Unit I. (The directions are repeated here for convenience.)

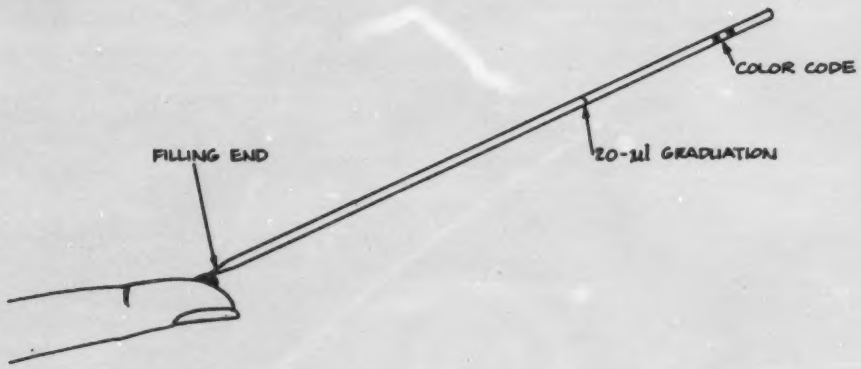
Choose someone to puncture your finger for you, unless you wish to do it yourself. (Note that most of the following instructions are for the person making the puncture.) First, examine--do not open--the package containing the sterile blood lancet. One end should read "open this end" or "peel to open." It is most important that you open that end, since it is the "handle" end. The opposite end is the blade, which you should not touch in order to keep it sterile.

The best finger to puncture is the one that gets the least use. This is usually the left ring finger (or the right ring finger for left-handed persons). Before the puncture is made, it is important to cleanse the skin. To do this, open the alcohol cotton wipe package. Then hold the finger with one hand and scrub its tip thoroughly with the alcohol wipe.

Open the blood lancet package. Make a quick, firm puncture with the lancet in the tip of the finger, using a rapid in-and-out motion. If blood does not flow immediately, quickly repeat the puncture. Dispose of the lancet as directed by your instructor. Used lancets are contaminated and can cause infection if reused by someone else.

5. Touch the filling end of your micropipet (see figure on the following page) to your puncture and allow the blood to rise up into the pipet until the meniscus is above the 20-µl graduation. (Note: If you are having trouble obtaining enough

blood, place your thumb above the puncture wound and apply gentle pressure downwards towards the wound. If you still cannot get blood, repeat Step 4 using a fresh sterile lancet.)



6. Cover the color-coded opening of the pipet with your finger, and remove the pipet from the puncture.

7. Adjust the blood level in the pipet to the calibration line by touching the filling end of the micropipet to a wad of clean cotton or tissue until the meniscus is at the 20-µl mark.

8. Wipe off the outside of the pipet and discharge the blood into cuvet A. Gently blow air through the pipet to facilitate its emptying.

9. Rinse the pipet 3 to 4 times by carefully drawing the EDTA-Na_4 blood solution in cuvet A up into the pipet and gently blowing it out again each time. This is done to ensure a complete transfer of the blood to the EDTA solution.

10. Standardize the colorimeter at 100% transmittance using the EDTA-Na_4 blank (cuvet B).

11. Find and record the percentage transmittance for the cuvet containing your blood sample (cuvet A).

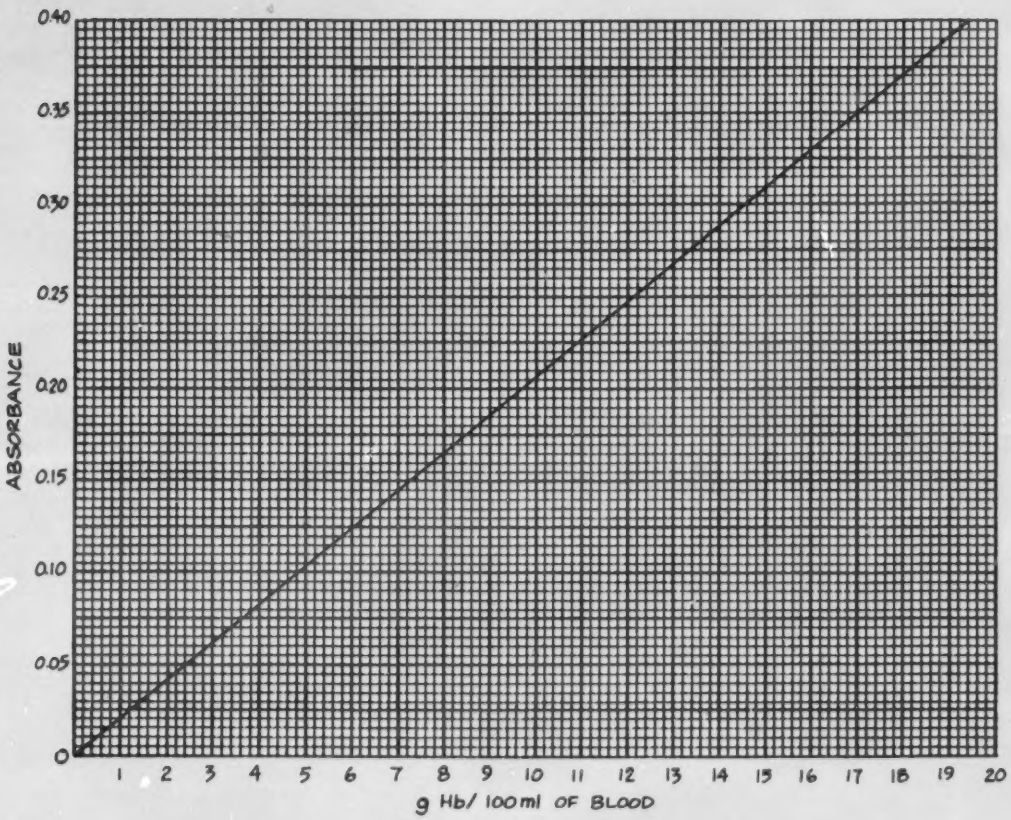
12. Determine the corresponding absorbance.

13. Use the standard graph (on the next page) to convert the absorbance of your blood sample into hemoglobin concentration in g Hb/100 ml of blood. Record the value.

DISCUSSION QUESTIONS:

1. How do your results compare with the values given for anemia in the introduction to the procedure?

2. Does there seem to be any consistent difference between the values for males and those for females?



LABORATORY ACTIVITY 11:

MICROSCOPIC EXAMINATION OF BLOOD SMEARS

INTRODUCTION:

Laboratory analysis of blood is used in almost all phases of medical diagnosis. One valuable procedure is microscopic examination of a blood smear. A great deal of information can be obtained in this manner. For example, the presence of abnormal cells in the blood, variations in the structure of blood cells and abnormal numbers of blood-cell types may be detected. All of these factors are of help in diagnosing various diseases.

In Part I of this activity you will prepare a blood smear and stain it with Wright's solution. This technique will enable you to observe erythrocytes and different kinds of leukocytes. Then in Part II you will compare a normal blood smear with various prepared smears of abnormal blood taken from persons suffering from a variety of diseases.

At this time you will also need to collect a small amount of blood and store it in heparinized (anti-clotting) tubes. The blood will be allowed to stand so that the cells settle, and the plasma will be used in Laboratory Activity 13. The procedure for collecting the blood sample is described in Part III of this activity.

PART I: PREPARATION AND OBSERVATION OF A BLOOD SMEAR

MATERIALS:

95% ethyl alcohol
dropper bottle of Wright's stain
dropper bottle of pH 6 solution
sterile blood lancet
alcohol cotton wipe packet
2 microscope slides
beaker, 50-ml
Petri dish cover or bottom
microscope
lens paper
lint-free cloth (optional)

PROCEDURE:

1. One important requirement for a good smear is a clean slide. Wash each slide in soap and water and then rinse well. (NOTE: HANDLE THE CLEAN SLIDES ONLY BY THEIR EDGES TO PREVENT MARKING THEM WITH FINGERPRINTS.)

2. Dip each slide into 95% ethyl alcohol, and wipe it dry with a lint-free cloth. If a lint-free cloth is not available, hold the slide until it dries. You can speed drying by holding the slide vertically while touching one end to a paper towel.

3. Place the clean slides on a paper towel.

4. Puncture your finger as you did in Laboratory Activity 10. Use the alcohol swab to wipe away the first drop.

5. Squeeze your finger until a small drop of blood forms. Touch this drop lightly at one end of the slide as shown in Figure 1. Do not touch the slide with your finger. (A small drop is preferred because too much blood on the slide will result in a smear that is too thick; and the blood cells will overlap each other, making microscopic observation difficult.)

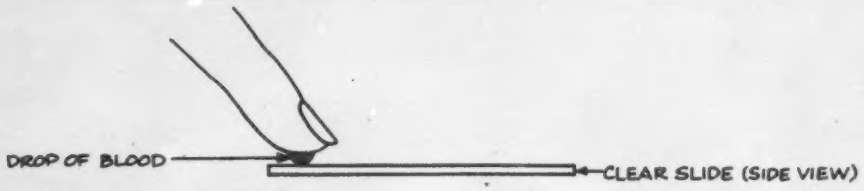


FIGURE 1: Touching the drop to the slide.

6. Take a second clean slide and place one of its ends on the middle of the first slide at an angle of about 45° over the drop of blood (Figure 2).

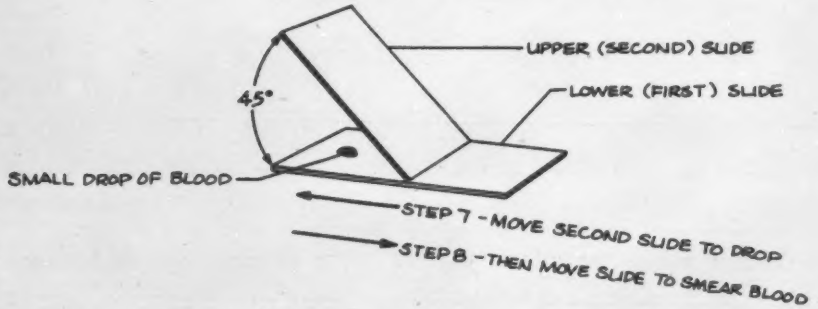

UPPER (SECOND) SLIDE
LOWER (FIRST) SLIDE
45°
SMALL DROP OF BLOOD
STEP 7 - MOVE SECOND SLIDE TO DROP
STEP 8 - THEN MOVE SLIDE TO SMEAR BLOOD

FIGURE 2: Spreading the drop.

7. Without changing the angle, move the upper slide along the surface of the lower slide toward the blood until it just makes contact with the blood.

8. When the blood has spread along the entire edge of the upper slide, push the upper slide in the opposite direction, away from the drop of blood. This will distribute the blood in a thin layer over the surface of the lower slide. (A good smear is one in which the cells are evenly distributed and not so numerous as to overlap. The smear should be as wide as the slide and cover only one-half to two-thirds the length of the slide as shown in Figure 3. If there is too much blood, the smear will cover the whole length of the slide and the cells will be too numerous to observe properly. If this happens, you will have to start all over again.)

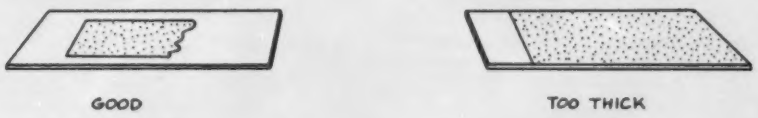


FIGURE 3: A good smear and a bad smear.

9. Dry the slide by passing it back and forth through the air.

10. Place a beaker on a glass Petri dish top or bottom (Figure 4). (The Petri dish will be used to catch any of the stain that might spill.) Position the slide on the beaker, as shown in Figure 4.

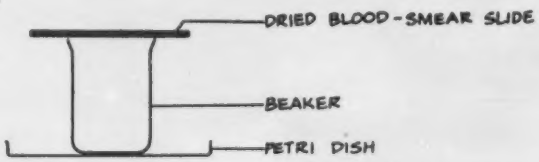


FIGURE 4: Staining set-up.

11. Apply enough Wright's stain with a dropper to just cover the surface of the slide. Allow this to stand for about 3 minutes (CAUTION: WRIGHT'S STAIN STAINS CLOTHING, SKIN, TABLETOPS, ETC.)

12. Dilute the Wright's stain on the slide by adding as much of the pH 6 solution with a dropper as will remain on the slide without overflowing. Allow this to stand for about 10 minutes.

13. Remove the stain from the slide by gently washing with a few droppers full of distilled water. Tilt the slide very slightly to allow any debris to wash off the slide. (When washing the slide keep it over the beaker, to avoid staining the tabletop.)

14. Wipe off the underside of the slide with a paper towel and allow the slide to air dry.

15. Place the stained smear on the stage of your microscope and examine it first at low power, then at high power.

16. Find examples of erythrocytes and leukocytes and compare them with regard to size, shape, color after staining, quantity, etc. (You may wish to refer to the diagrams of these cells in Sections 10 and 11.) Record diagrams and notes on a data sheet. (Note: The blood components are more readily seen in those sections where the blood smear is thinnest.) You may also observe some platelets.

17. Examine three or four different fields of cells and estimate the ratio of erythrocytes to leukocytes.

18. Save the slide that you have prepared until you have completed Part II.

PART II: EXAMINING SLIDES OF ABNORMAL BLOOD COMPONENTS

MATERIALS:

prepared slides of abnormal blood cells
microscope
lens paper

PROCEDURE:

A few prepared slides showing blood abnormalities are available. Observe each of these, and compare the appearance of the blood cells with those in the smear you prepared or with a picture of normal blood cells. Record the name of the abnormality and any distinguishing characteristics.

PART III: PREPARATION FOR LABORATORY ACTIVITY 13

MATERIALS:

alcohol cotton wipe packet
sterile blood lancet
micropipet, heparinized, 20-µl
silicone rubber cement
masking tape

PROCEDURE:

1. Puncture your finger as in Part I of this procedure. Wipe away the first drop.

2. Touch the filling end of the heparinized micropipet to the drop of blood. Allow the blood to rise up into the pipet until it reaches the colored line about three-fourths of the way up the tube. You may have to orient the pipet horizontally to fill it. You may also have to apply gentle pressure by squeezing the finger to obtain the needed blood.

3. When you have collected the blood, cover the opening at the color-coded end of the tube with a finger to keep it from dripping, and remove the pipet from the puncture.

4. Wipe the outside of the pipet with absorbent paper, and seal both ends with dabs of silicone rubber cement.

5. Label a piece of tape with your initials, place it around the pipet and store vertically in the refrigerator.

DISCUSSION QUESTIONS:

1. Why was it important to have a clean slide and clean microscope lens in this activity?

2. Why was it important to minimize the amount of blood on the slide in Part I?

3. How might you improve your chances of getting an excellent smear?

4. How did your stained blood smear differ from the prepared slides?

LABORATORY ACTIVITY 12:

ELECTROPHORESIS OF AMINO ACIDS

GENERAL INTRODUCTION:

You have already been exposed to a number of different techniques for separating different substances in mixtures and have used some of these techniques in the laboratory. For example, you separated fats from protein for nutrient analysis by extracting the fats with Freon. In this activity, you will explore a new separation technique called "electrophoresis," which involves the movement of charged particles through a liquid medium in response to an electric current.

This description could apply just as well to electroplating. In fact, you may find it useful to review some of the concepts and terminology discussed in the introduction to the electroplating activity (Unit I, Activity 22). Just as in electroplating, the medium used in electrophoresis is an ionic solution capable of carrying electric current. The difference is that in electrophoresis the charged particles move along on a strip of paper or other material that has been soaked in the medium. By placing chemical compounds on the strip and passing a current through the strip, the coupounds are caused to migrate. They move toward either the anode (+) or the cathode (-), depending upon their charge. In this way a mixture of ionic compounds possessing different charges can be separated.

Another useful property of electrophoresis is that compounds with different net charges also move at different rates. For example, a Na^{+} ion and a Ca^{++} ion tend to move at different rates. This permits use of electrophoresis to identify the various compounds in a mixture. For example, after electrophoresis, a substance with a net charge of -2 will be found nearer to the anode than a substance with a net charge of -1. Suppose you have a mixture containing two substances (x and y). You can separate x from y by electrophoresing the mixture. Furthermore, if the net charges of x and y are known, you will be able to identify the x and y spots by studying their positions on the electrophoretic strip after electrophoresis.

In recent years, electrophoresis has become an important method for detecting diseases that involve protein abnormalities, such as sickle-cell anemia. Biochemists also use this method to study enzymes and other proteins.

Both this activity and the next involve the separation of chemicals by electrophoresis. First, two amino acids possessing different net charges will be electrophoresed to demonstrate the method and give you some practice with the laboratory techniques involved. Then in Activity 13 the plasma portion of the blood samples that were drawn in Activity 11 will be electrophoresed in an attempt to separate blood proteins.

PART I: ELECTROPHORESIS OF AMINO ACIDS

INTRODUCTION:

The first portion of this activity involves (1) setting up the apparatus for electrophoresis, (2) preparing the samples of amino acids to be electrophoresed and (3) performing the actual electrophoresis.

Two beakers are used, each containing an ionic solution, in which electrodes are immersed (see Figure 1). The two solutions are bridged by a strip of electrophoresis paper that has been soaked in the solution. Remember that the solution that permeates the strip is ionic so when a voltage is applied across the two electrodes, current flows between the solutions in the two beakers through the electrophoretic strip.

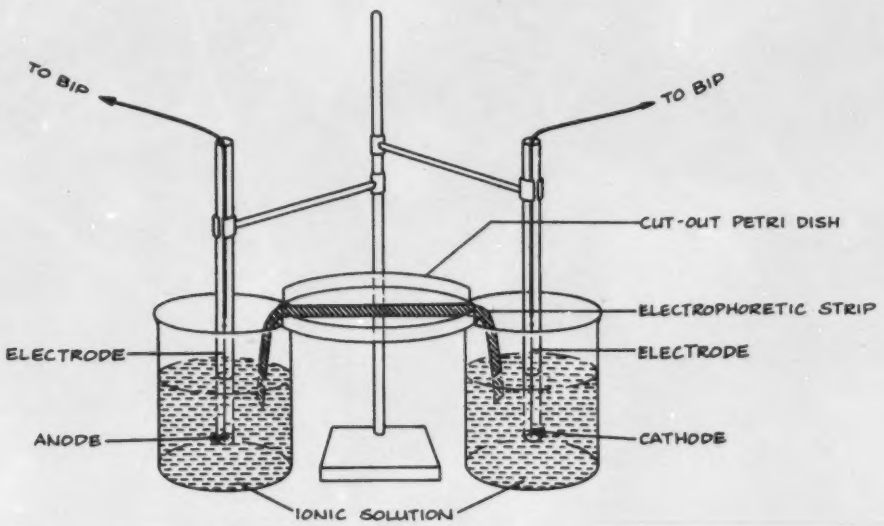
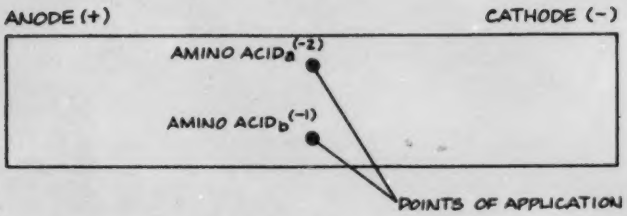


FIGURE 1: Electrophoresis set-up.

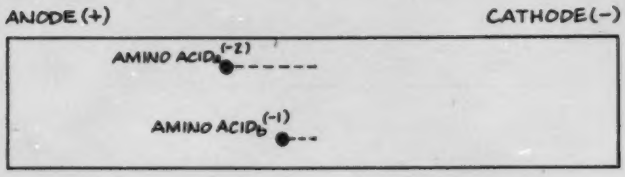
The amino acid samples are placed on the electrophoretic strip by applying small drops of amino acid solution with a capillary tube. When current flows through the wet strip the amino acids will migrate. The direction and distance of migration depends upon their net charges and to some degree on their masses. For example, suppose one amino acid possesses a net charge of -2 and the other a net charge of -1 and they are both of the same mass. Both amino acids will migrate toward the anode (the positive electrode), and the amino acid possessing a charge of -2 will migrate a greater distance from its point of application. (See Figure 2 on the following page.)

MATERIALS:

electrophoretic strips (in bath of ionic solution)
ionic solution, pH 9.1
2 amino acid solutions
2 capillary tubes
2 beakers, 150-ml
2 microscope slides
"cut-out" plastic Petri dish
Petri dish cover
BIP
2 electrophoresis electrodes
ring stand with 2 clamps
paper tissues
glass-marking pencil



A. Before electrophoresis.



B. After electrophoresis.

FIGURE 2: Electrophoresis of amino acids of different charge.

PROCEDURE:

1. Fill each of two 150-ml beakers with approximately 120 ml of ionic solution. The exact amount is not critical, but the height of the two liquid levels should be the same.

2. Clamp the two electrodes to the ring stand and immerse the ends in the solutions as shown in Figure 1. Separate the beakers by about 5 cm so that a Petri dish can rest securely across the edges of the two beakers.

3. Attach the electrode leads to terminals Y and Z on the BIP. Either electrode may be attached to Y or Z--it does not make any difference. You should be aware, however, that the electrode attached to Z will be the anode (+), while the Y electrode will be the cathode (-).

(Note: From Step 4 on, plan to work quickly until you complete Step 11. Otherwise, the amino acid solutions may dry out. You may want to read ahead before proceeding, so that you will know what you have to do in the next few minutes.)

4. Two amino acid solutions are available. They are labeled A and B. Place one drop of each solution on a microscope slide, as shown in Figure 3. Be sure to label each drop correctly.

FIGURE 3: Preparing the amino acid samples.

5. Obtain an electrophoretic strip that has been soaking in the ionic solution. Place it lengthwise over a clean microscope slide, on a paper towel. (Handle the strip very carefully since it tears easily. Also handle it only near the ends.)

6. Blot the strip lightly with a paper tissue. (Don't overdo it. If the strip is too dry, current will not flow through it. On the other hand, there should not be any "pools" of ionic solution on the surface of the strip.)

7. Use a pencil to mark the electrophoretic strip as shown in Figure 4. The samples of amino acids will be applied below the "A" and above the "B" on the strip, respectively. The "+" and "-" marks will be used to orient the strip properly between the electrodes in the electrophoresis set-up.

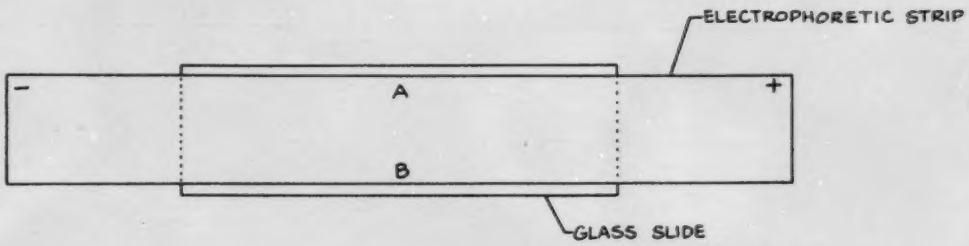


FIGURE 4: Marking the electrophoretic strip.

8. Place the end of a capillary tube in the drop of amino acid solution labeled A. The liquid should rise inside the tube.

9. Apply Solu on A 0.5 ± 0.1 cm directly below the "A" you marked on the strip. The sample is applied by simply touching the pipet to the strip. A very small amount will flow onto the paper. Don't be concerned if you are unable to see the sample as it flows onto the strip. Only a small amount (about ten millionths of a liter) is needed. (Note: This step is important. If too much solution is applied and/or the drops overlap, the results will be poor.)

10. Repeat Steps 8 and 9 for amino acid solution B, but this time place the sample 0.5 ± 0.1 cm directly above the "B" marked on the strip. (Be sure to use a clean capillary tube.)

11. Place the electrophoretic strip on the Petri dish as shown in Figure 5.

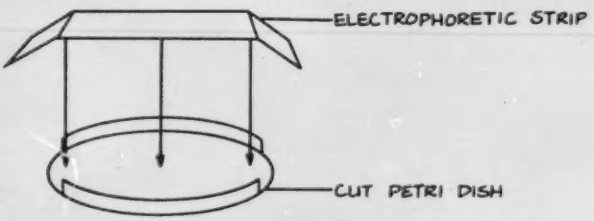


FIGURE 5: Positioning the electrophoretic strip.

12. Place the cover of another Petri dish over the Petri dish containing the electrophoretic strip and position the set-up between the beakers as shown in Figure 1.

Make sure the end of the strip marked "+" is suspended in the beaker of solution connected to the anode, "Z".

13. Plug in the BIP and allow the samples to electrophorese for 45 minutes.

PART II: DEVELOPING THE ELECTROPHORESED AMINO ACIDS

INTRODUCTION:

At this point you have completed the electrophoresis of the amino acid samples, but the amino acids cannot be seen. In order to determine how far the samples have migrated, it is necessary to make the amino acids visible. This "developing" of the strip is done by exposing it to a series of chemicals. First the strip is exposed to a mixture that chlorinates the amino acids. After this, the strip is dipped in a mixture containing a chemical called "o-tolidine", which reacts with the chlorinated amino groups causing the amino acids to form dark, visible compounds.

MATERIALS:

- electrophoretic strip (from Part I)
- HCl solution
- $KMnO_4$ solution
- developer bath
- 2 acetic acid baths
- quart jar, with top
- graduated cylinder, 100-ml
- length of wire (approximately 30 cm)

PROCEDURE:

1. After the electrophoresis is complete, unplug the BIP and remove the electrophoretic strip from the apparatus. Place the strip on a clean paper towel.

2. Starting at the end of the strip marked "-", wrap the strip around a piece of wire a few times so that you can hold the wire up in the air without the strip falling off (Figure 6).

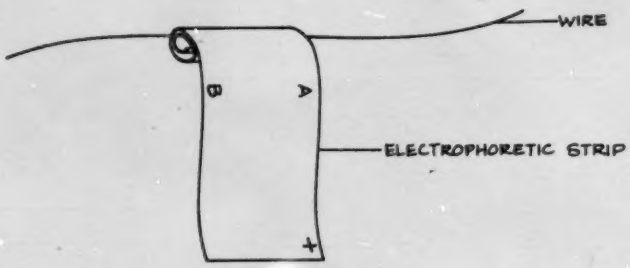


FIGURE 6: Suspending the electrophoretic strip.

3. Prepare the chlorinating mixture by obtaining 50 ± 1 ml each of the HCl and $KMnO_4$ solutions and pouring them into the quart jar. As soon as you have done this, cover the jar to prevent any chlorine gas from escaping. (CAUTION: CHLORINE GAS IS POISONOUS. KEEP THE JAR CLOSED AT ALL TIMES EXCEPT WHEN INSERTING AND REMOVING THE STRIP. IF HOODS ARE AVAILABLE, WORK UNDER ONE.)

4. Suspend the strip above the chlorinating mixture, and close the jar (Figure 7 on the following page). (The strip should not touch the mixture.) Leave the strip in the jar for 5 minutes.

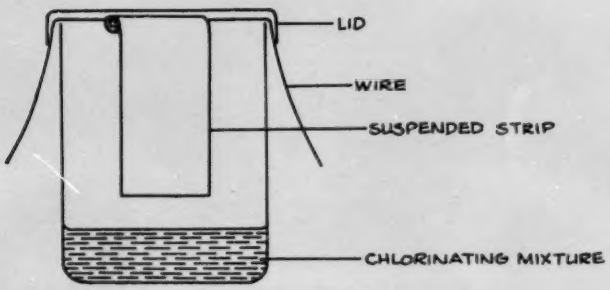


FIGURE 7: Placing the strip in the chlorinating chamber.

5. At the end of 5 minutes, remove the strip from the jar and dip it in the developer solution.

6. Rinse the strip in the two acetic acid baths and set it on a paper towel to dry.

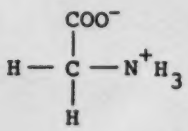
7. Draw penciled circles around the amino acid spots. It is necessary to mark the spots because the colors may fade.

DISCUSSION QUESTIONS:

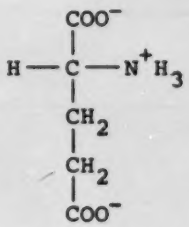
1. Did amino acid A migrate toward the anode or the cathode? What about amino acid B?

2. Did the two amino acids migrate the same distance from their points of application? If not, what does this suggest about the differences between these two amino acids?

3. The amino acids that you electrophoresed possess the following structures.



glycine



glutamic acid

Which functional groups present in amino acids account for the migrations noted?

4. Consider the structures of the amino acids (Question 3) and the results of your electrophoresis activity. Can you figure out which amino acid corresponds to sample A and which to sample B? Explain your reasoning.

5. Why was it necessary to label the strip "+" and "-" at either end?

LABORATORY ACTIVITY 13:

ELECTROPHORESIS OF PLASMA PROTEINS

INTRODUCTION:

In Activity 12 it was demonstrated that two amino acids possessing different net charges could be distinguished by studying their migration patterns after electrophoresis. In this activity the same technique is applied to demonstrate the presence of proteins in blood plasma. Proteins separate during electrophoresis for the same reason as amino acids--they possess different net charges and masses. Recall that proteins are made up of amino acids of various kinds and in varying proportions.

Though plasma contains many proteins, it will only be possible to distinguish between two groups of plasma proteins with the available equipment: the albumin and globulin fractions. (The clinical value of plasma protein electrophoresis is discussed in Section 13.)

The same set-up and ionic solution are employed in this activity as you used for the electrophoresis of amino acids in Activity 12. However, a different "developer" will be used which is more specific for proteins. (The reagent used to detect amino acids in the preceding activity does not stain proteins readily.)

PART I: ELECTROPHORESING THE PLASMA PROTEINS

MATERIALS:

electrophoretic strips (soaking in ionic solution)
ionic solution
albumin solution
gamma globulin solution
blood samples drawn in Activity 11
2 beakers, 150-ml
2 capillary tubes
BIP
2 microscope slides
ring stand and 2 clamps
"cut-out" plastic Petri dish
Petri dish cover
2 electrophoresis electrodes
paper tissues
mm ruler

PROCEDURE:

1. Prepare the electrophoresis set-up as you did in Activity 12.

2. In addition to the blood plasma, you will be spotting the electrophoretic strip with two standard solutions--one of albumin and one of gamma globulin. (A comparison of their movement during electrophoresis with the movement of the plasma proteins will be of help in identifying electrophoresed plasma protein fractions.) Obtain a drop or two of each of these two solutions on a microscope slide. Be sure to label the location of each solution on the slide.

3. Obtain an electrophoretic strip that has been soaking in ionic solution. Place it lengthwise over a clean microscope slide, on a paper towel.

4. Blot the strip lightly with a paper tissue and mark it lightly with a pencil as shown in Figure 1 on the following page. (Note: Make the "A" and "G" marks small so that they do not interfere with the electrophoresis of the plasma proteins.)

FIGURE 1: Marking the electrophoretic strip.

5. Use a capillary tube to apply the sample of the albumin solution to the strip. The point of application should be about 5 mm directly below the "A" marked on the strip.

6. Repeat Step 5 for the gamma globulin solution, but this time apply the sample about 5 mm above the "G" on the strip. (Be sure to use a clean capillary tube.)

7. In order to apply the plasma sample, you will need to unplug the heparinized tube containing the blood sample taken during Activity 11. The plasma is the clear fluid at the top ("color-coded" end) of the tube. The cells found in the blood have settled into the lower (red) fraction at the bottom. Peel the silicone cement from the end where the red blood cells have collected (see Figure 2). Once the cement is removed, hold your finger firmly over the open end and peel away the cement from the other end containing the plasma (clean fluid).

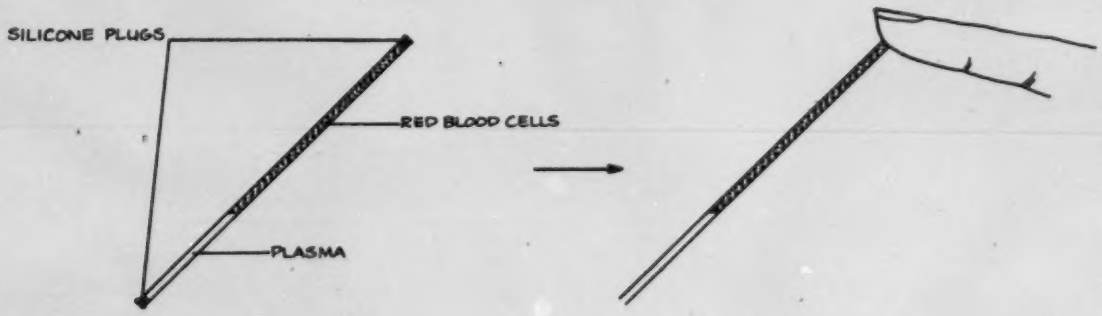


FIGURE 2: Unplugging the capillary tube.

8. To apply the sample of plasma, place the plasma end of the tube on the electrophoretic strip midway between the albumin and gamma globulin samples (Figure 3 on the following page). Quickly remove your finger from the other end of the tube and then replace it. Remove the tube from the strip. This technique will allow only a small volume of plasma to leave the tube and deposit on the strip. (Note: It is important that only a small spot of plasma be applied. To accomplish this, it is crucial that you remove your finger from the upper end of the tube for only a fraction of a second.)

9. When you have applied the plasma, the three samples should be lined up as shown in Figure 3, and none of them should be touching each other. If one of the spots you applied was large and you think it might have spread into another sample

spot, obtain another electrophoretic strip and start again.

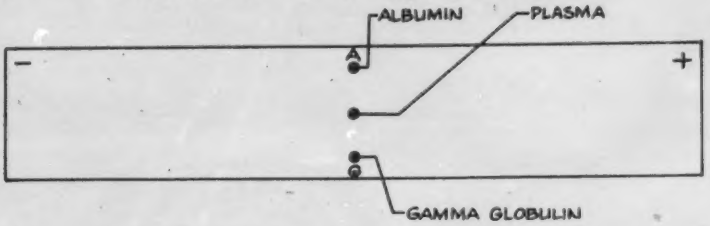


FIGURE 3: Position of spotted samples.

10. Place the electrophoretic strip in the "cut-out" Petri dish as you did in Activity 12. Place the cover of another Petri dish over it, and position the strip between the beakers of the electrophoresis set-up. Remember to make sure that the end of the strip marked "+" is suspended in the beaker of solution connected to the anode (Z).

11. Plug in the BIP and allow the samples to electrophorese for one hour.

PART II: DEVELOPING THE ELECTROPHORESED PLASMA PROTEINS

INTRODUCTION:

The stain used to develop the plasma proteins after they have been electrophoresed is Ponceau S dye. This red dye binds to proteins. By dipping the strips in the dye and then rinsing them in acetic acid, all the dye is removed that is not bound to proteins on the electrophoresed strip. Thus, the red spots will correspond to proteins.

MATERIALS:

Ponceau S dye 2 acetic acid baths

PROCEDURE:

1. After the proteins are electrophoresed for one hour, unplug the BIP, remove the strip from the apparatus and place it on a clean paper towel.

2. Use the Petri dish cover which was used in the electrophoresis set-up to obtain some Ponceau S dye.

3. Pick up the electrophoretic strip by the ends and hold it in the Ponceau dye solution so that the mid-section of the strip is immersed. The strip should remain in the dye for two minutes.

4. Remove the strip from the dye and dip it in one of the two acetic acid baths and then the other. When finished, the strip should be almost white except for the red spots which show the positions of the proteins.

5. Set the strip aside on a paper towel to dry. (If you wish to save the strip, place it between two pieces of paper towel and place it under a heavy object to press it flat.)

DISCUSSION QUESTIONS:

1. Is the net charge on the albumin proteins negative or positive? On the gamma globulin proteins?

2. What were the results of electrophoresing the plasma proteins?

3. Can you identify the albumin and gamma globulin fractions of the electrophoresed plasma proteins? Which is which?

ACTIVITY 14:

IMMUNITY

INTRODUCTION:

The purpose of this activity is, in part, to introduce the subject of immunology, which is very important in modern medicine. Immunology has applications ranging from preventing disease to transplanting kidneys.

The text of this activity has been divided into chapters. In this short chapter and in the ones that follow, you will read some accounts tracing the development of theories of immunity. We would like to put you into a "time machine" and carry you back to a time when new and revolutionary ideas were being considered about our body defenses against infectious disease. We want you to imagine that you are a scientist making decisions, designing experiments and evaluating observations. This brings us to a second goal of this activity--to provide some insight into scientific approaches to problems.

Your instructor will assign you to a small group or team and ask you to read Chapter 1. After you and your team read the chapter, discuss the question at the end. Make an attempt to solve the problem within your group and bring a short, written answer to the instructor. The instructor will then provide you with Chapter 2. Repeat the cycle of reading, discussing and answering the questions with Chapter 2, then Chapter 3, and so on until you finish the last chapter.

CHAPTER 1

Eli Metchnikoff began his scientific life as a marine biologist. And he might have continued in that occupation indefinitely but for a chance event in 1882 on the island of Sicily. He had gone with his family to the scenic Strait of Messina where they might happily vacation while he collected and studied the small creatures that lived in the waters nearby. It was that study that one day changed him from marine biologist to bacteriologist.

"One day, as the whole family was at the circus to see some trained apes," reported Metchnikoff, "I remained home alone with my microscope." To Metchnikoff, it appears, trained apes were less fun to watch than starfish larvae, for that is what he set on the stage of his microscope.

The larva (immature form) of the starfish is a good subject for microscopic study. Among the interesting sights that can be seen within its transparent body are small wandering cells--cells that look like amebas.

As Metchnikoff watched these cells in their aimless wanderings, he wondered what these cells were doing in the larva. What could their function be? Somehow it

occurred to him that they might serve the larva in a unique way. Such cells, he decided, might "function to protect the organism against intruders."

Metchnikoff was so excited by his new idea that he couldn't wait to test it out. He went out to a garden where he often went to think. As he walked among the flowering plants and fruit trees, his hand brushed against a rosebush, leaving several small scratches and a thorn in his hand. He looked at the thorn penetrating his skin and thought of a microbe entering his body. Metchnikoff rushed from the garden back to his microscope with a possible solution for testing his new idea. He reached for his culture of starfish larvae.

Question: What sort of experiment might Metchnikoff be preparing to perform to help answer his question about the role of wandering cells?

LABORATORY ACTIVITY 15:

DETERMINING ANTIBODY TITERS IN SIMULATED SERUM

INTRODUCTION:

In Section 14 antibodies were defined as proteins synthesized by the body that function to destroy "foreign" substances that are harmful to the body. The foreign substance which causes lymphocytes to produce antibodies, and with which an antibody reacts, is called an "antigen." Antibodies react with antigens in a manner very similar to the way enzymes react with their substrates.

One interesting characteristic of antigens and antibodies is that either one alone may not be visibly detectable in a solution, but when they react to form an antibody-antigen combination, the combination precipitates out of solution and becomes visible. Scientists have made use of this phenomenon to determine whether or not, the antibody for a specific antigen is present in an individual's serum. If there is antibody present, the "titer" or amount can also be estimated.

For example, suppose a blood bank is conducting a "blood drive," and they want to make sure that no donors have had hepatitis recently (because it is infectious). By mixing hepatitis virus (which contains the antigens) with a sample of the prospective donor's serum, they can tell whether a person possesses antibodies to hepatitis. A precipitate of the two solutions would indicate that antibodies are present. If he does have antibodies in his serum, then he either has hepatitis now or has had it recently. This would disqualify him as a donor becuase his blood might contain live viruses.

In this activity you will be presented with a simulated serum sample that contains an unknown amount of antibodies to serum hepatitis. You will also be given a simulated antigen solution containing "serum hepatitis virus." It will be your task to determine the titer of antibody in the simulated serum sample. This is done by mixing a specific amount of antigen sample with samples of simulated serum diluted in varying amounts and noting the presence or absence of an antibody-antigen reaction. (In this case, a white precipitate indicates that a reaction has occurred.) By de-

termining the dilution at which the antibody-antigen reaction is no longer detectable, you will estimate the amount of antibody in the undiluted serum sample. This value is an approximation of the titer.

MATERIALS:

simulated serum sample
simulated antigen sample
9 test tubes, 13 x 100 mm
graduated cylinder, 10-ml
3 rubber stoppers, #00
pipet, 10-ml
6 pipets, 1-ml
test-tube rack
glass-marking pencil

PROCEDURE:

1. Use the graduated cylinder to add 5 ml of simulated antigen to a test tube labeled "A" and 5 ml of simulated serum sample to a test tube labeled "S". (Make sure you rinse the cylinder between transfers.)

2. Label three test tubes "10 S," "100 S" and "1000 S." These tubes will be used to make the diluted serum samples. Tube 10 S will be for a serum sample diluted by a factor of ten with water, and so on. (The symbols "S" and "A" will represent "serum" and "antigen," respectively.)

3. Add 4.5 ± 0.1 ml of water each to Tubes 10 S, 100 S, and 1000 S.

4. Using a 1-ml pipet, add 0.5 ± 0.01 ml of undiluted serum sample to Tube 10 S. Rinse the pipet by drawing liquid back up into the pipet and releasing it at least five times. Then stopper the test tube and agitate.

5. Using the same pipet as in Step 4, transfer 0.5 ± 0.01 ml of the diluted sample in Tube 10 S to Tube 100 S and mix.

6. Transfer 0.5 ± 0.01 ml of the sample from tube 100 S to tube 1000 S and mix.

7. Label another set of test tubes "0 SA," "10 SA," "100 SA" and "1000 SA." These tubes will be used to test for the formation of an antibody-antigen combination in Step 10.

8. Use a clean pipet to transfer 0.5 ± 0.01 ml of simulated antigen sample into each of the four test tubes.

9. Now you are ready to "layer" the simulated serum sample over the antigen sample. This is done by tilting the test tube slightly and pipetting the serum sample onto the wall of the test tube (Figure 1). In this way the sample is allowed to flow slowly down the wall of the tube to form a

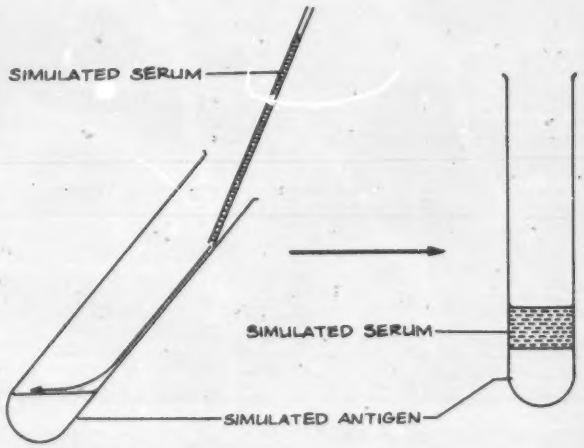


FIGURE 1: How to layer the serum over the antigen.

layer on top of the antigen sample. For best results, try to release the serum sample from the pipet slowly.

10. Using a clean pipet and the procedure described in Step 9, layer 0.5 ± 0.01 ml of undiluted serum sample into tube 0. When you have completed the transfer, right the tube gently (so as not to mix the layers) and observe whether you can detect any precipitate between the two layers. A white precipitate indicates a positive test, the absence of precipitate a negative test (see Figure 2). Make a note of the results.

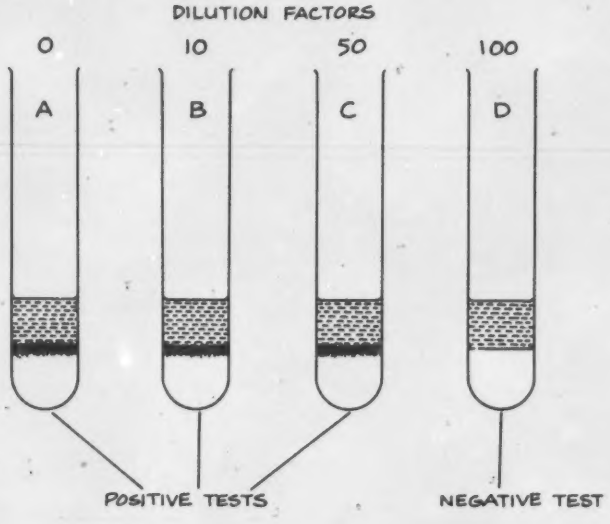


FIGURE 2: Positive and negative antibody-antigen tests.

11. Repeat Step 10 using the diluted serum samples in tubes 10 S, 100 S, and 1000 S by layering them into the appropriate test tubes containing simulated antigen. Use a separate pipet to transfer each sample. Note your results.

DISCUSSION QUESTIONS:

1. The titer of antibodies present in a serum sample is recorded as the dilution factor of the most concentrated serum sample that gave no antibody-antigen reaction. For example in Figure 2, if tube D corresponds to a dilution factor of 100, then the titer of the serum sample would be 100. What is the titer of antibodies in your serum sample?

2. If you were conducting a blood drive, would the "blood" from which your serum sample was obtained be acceptable? (Assume that any titer other than zero is unacceptable.)

3. Can you think of any way in which antibody titers might be of use in diagnosing a disease when a person has symptoms characteristic of several diseases?

4. Using the dilutions given in this procedure, you can give only a rough estimate of antibody titer. How could you "pinpoint" the titer value of your serum sample more accurately?

5. Explain why a titer of 10,000 indicates the presence of 100-fold more antibodies than one of 100.

LABORATORY ACTIVITY 16:

BLOOD CLOTTING AND COAGULATION TIME

GENERAL INTRODUCTION:

Section 16 discussed some of the ways in which the body acts to stop bleeding from a wound. These included constrictions of blood vessels, aggregation of platelets, and the formation of fibrin clotting at the site of the wound. In this activity you will have an opportunity (1) to observe microscopically the fibrin network that forms when blood clots and (2) to determine your coagulation time (the time it takes for your blood to clot).

PART I: MICROSCOPIC OBSERVATION OF FIBRIN

INTRODUCTION:

Blood clotting involves the formation of an insoluble blood protein called "fibrin." Fibrin molecules unite to form a network of protein strands which hold blood cells in place, preventing them from leaking out of a wound. The aggregate formed by these millions of fibrin molecules is called a blood clot.

Long before a blood clot becomes visible to the naked eye, the formation of the fibrin network can be observed using a microscope. By puncturing your finger and examining a drop of blood under the microscope you will be able to observe the fibrin network develop over a period of time.

MATERIALS:

sterile blood lancet
alcohol cotton wipe packet
glass slide
microscope
glass-marking pencil

PROCEDURE:

1. Mark a small "X" near one end of a microscope slide. Focus on the X with the 40X objective of the microscope. Carefully remove the slide, leaving the microscope in focus. (This is done so that you will not have to focus the microscope when you view the blood. No coverslip is used in this procedure and any further focusing could cause blood to stick to the objective.)

2. Puncture your finger and place a drop of blood on the slide away from where you marked the X. (Do not add a coverslip.) Quickly place the slide on the microscope stage and observe the blood near the edge of the drop. Watch for the formation of threads of fibrin. Work quickly, for the first stages of clotting may occur within seconds. Remember not to change the focus.

3. Observe the drop of blood for the next five minutes. Make sketches and describe the developments that you see.

PART II: DETERMINING COAGULATION TIME.

INTRODUCTION:

Coagulation time is the time it takes for blood to coagulate (clump together or clot). This is directly related to the time necessary for the fibrin network to form.

Normally coagulation time is between two and six minutes. A prolonged coagulation time may be an indication of some blood disorders, such as hemophilia. You will determine your coagulation time by drawing a fresh sample of blood into a capillary tube and noting the time that elapses until a strand of coagulated blood (see figure) is observed.

Strand of coagulated blood

MATERIALS:

alcohol cotton wipe packet
sterile blood lancet
2 capillary tubes
clock with second hand

PROCEDURE:

1. Puncture your finger, and wipe away the first drop of blood with the alcohol swab. Record the time that the finger was punctured.

2. Quickly fill at least one capillary tube, preferably two, with blood. In general, letting the arm hang down will lead to release of enough blood for the activity. It may be necessary to "milk" the finger to get out a few extra drops of blood. This is done by alternately squeezing and releasing pressure on the finger at a point between the palm and the puncture.

3. Break off a one- to two-cm section of tubing every 30 seconds until a strand of coagulated blood can be seen (see Figure). Break the tubing just as you would break a toothpick. Be careful to pull the pieces of tubing apart slowly or you will break any fibrin strands that may have formed.

4. Determine the amount of time that passed before you observed a strand of coagulated blood. This is your coagulation time.

DISCUSSION QUESTIONS:

1. What happens on the microscopic level when blood clots? What happens on the molecular level?

2. Compare your results on coagulation time with the results obtained by others in your class. How much variation was there?

3. How did the red blood cells look as time passed? (Recall the shape of the RBC's in Laboratory Activity 11.) Can you think of any reason for the change in shape?

LABORATORY ACTIVITY 17:

EFFECTS OF ACIDS AND BASES ON THE pH OF SOLUTIONS

INTRODUCTION:

When an acid or base is added to water, the pH of the solution decreases or increases. If one were to graph the pH of the solution versus the amount of acid added to it, a curve similar to the one shown in Figure 1 would be the result.

Note that the pH of the solution changes in a "near-linear" fashion in response to the addition of the acid. If the starting liquid is a solution (rather than pure water), it will respond in a similar way to the addition of acid. However, the rate at which pH changes as acid is added depends on the solution. For example, consider the graphs showing the change in pH of two solutions, X and Y, with the additon of acid (Figure 2).

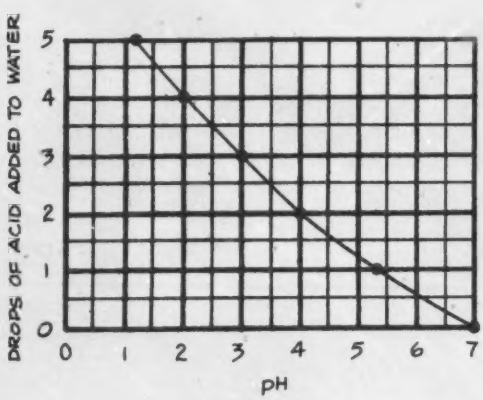


FIGURE 1

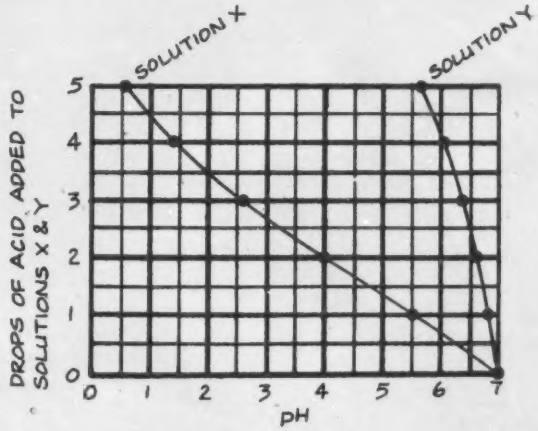


FIGURE 2

Note that the slopes of the graphs for Solutions X and Y are quite different. It is the slope that describes the amount of acid needed to change the pH by a given amount.

Certain solutions, such as Solution Y, change pH very slowly with the addition of acid or base. When the graph for such a solution is drawn, the curve is steep. Solutions which respond to the addition of an acid (or a base) in this manner are known as "buffers," and they play a very important role in homeostasis.

In this activity you will test a variety of solutions to determine how they respond to the addition of an acid or base (i.e., which ones are buffers). Then, in Section 17, you will consider why buffers respond as they do and why they are important to homeostasis.

MATERIALS:

1 M HCl solution
1 M NaOH solution
3 test tubes, 16 x 125 mm
4 beakers, 50-ml
1 M NaCl solution
1 M $NaHCO_3$ solution
2 medicine droppers
test-tube rack

1 beaker, 150-ml
pipet, 10-ml
3 pipets, 1-ml
glass-marking pencil
pH paper, Hydrion, pH 1 to 11
graph paper

PROCEDURE:

1. Place three test tubes in a rack and place a paper towel in front of them. Label the paper near the test tubes as shown in Figure 3.

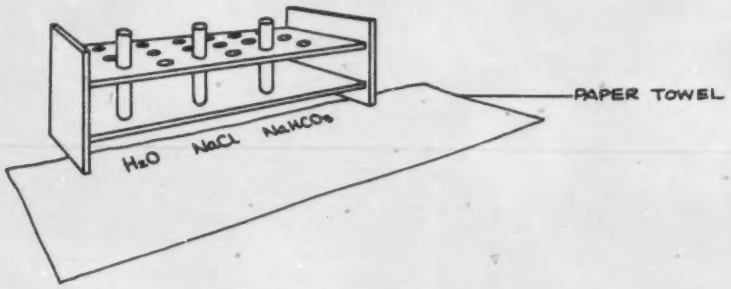


FIGURE 3

2. Label four beakers, "HCl," "NaCl," "NaOH" and "$NaHCO_3$" (sodium bicarbonate). Obtain about 20 ml of each of these four solutions. You will also need a beaker containing about 100 ml of water.

3. Transfer 10 ± 0.1 ml of water into the H_2O tube, and 9 ± 0.1 ml of water into both the NaCl and $NaHCO_3$ tubes.

4. Pipet 1 ± 0.01 ml of 1 M NaCl solution into the NaCl tube. Use the pipet to mix the solution and then leave it in the tube.

5. Use another 1-ml pipet to add 1 ± 0.01 ml of 1 M $NaHCO_3$ to the appropriate test tube. Again use the pipet for mixing the solution and then leave it in the test tube. Place a third 1-ml pipet in the test tube containing water.

6. To determine the pH of the solutions and to conserve pH paper, use the following procedure: Tear off strips of pH paper no longer than 1 cm. (Be sure your hands are clean and dry.) Place the pH paper strips at least 1 cm apart on a paper towel (see Figure 4). Use each pipet to transfer one small drop of solution to a

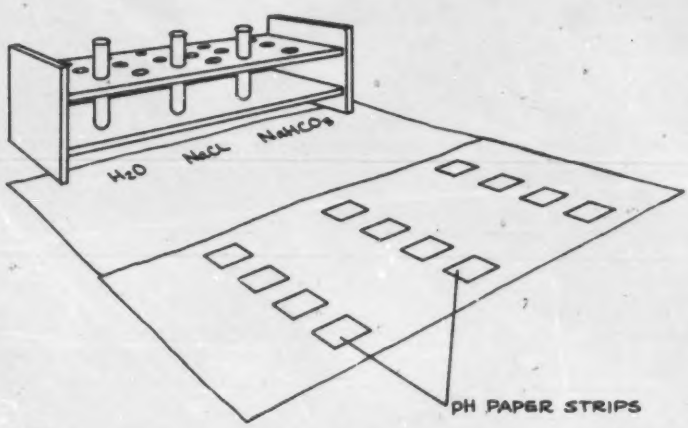


FIGURE 4

piece of pH paper. Be careful to return these pipets to the correct test tube. Refer to the color scale on the package of pH paper to determine the pH of each solution. Record your results.

7. The initial pH of the solutions may not be 7. If the pH's are outside the range of 6 to 8, add acid or base a drop at a time to bring them into this range. Record the adjusted pH, instead of the original pH.

8. Add one drop of 1 M HCl solution with a medicine dropper to each of the three test tubes. Drop the HCl directly into the solution (not down the side of the tube), so that all of it gets into the solution. Stir thoroughly.

9. Measure the pH of each solution and record the value on a data sheet similar to the sample data sheet shown.

SAMPLE DATA TABLE:

	pH of solution		
Drops of HCl added	H_2O	NaCl	$NaHCO_3$
0			
1			
2			
3			

10. Repeat Steps 8 and 9 until each solution has had 10 drops HCl added to it, or until it reaches a pH of 1, whichever comes first.

11. Discard the solutions in the test tubes, and rinse the test tubes and pipets thoroughly.

12. Repeat Steps 3 through 7 as before.

13. Repeat Steps 8 through 11, but this time add NaOH one drop at a time to the test tubes until 10 drops have been added or the pH reaches about 11, whichever comes first. Record your data for the addition of NaOH on a second data sheet.

DISCUSSION QUESTIONS:

1. From the data you have collected, construct a graph of "pH of Solution" (on the X-axis) vs. "Drops of HCl Added" (on the Y-axis). Construct a similar graph for "Drops of NaOH Added." (Note: Each graph will have 3 "curves" on it. Make sure you label each one appropriately.) Describe the responses of the three solutions in terms of the relative amounts of acid needed to change the pH by a given amount. Do the same for the addition of base. Which of the solutions is a buffer?

2. The slope of the graph provides a measure of the ability of a buffer to

resist a pH change. (Some buffers are more effective than others.) The strength of a buffer is called "buffer value." Buffer value is equal to the negative of the slope (when the Y-variable is "acid (or base) added" and the X-variable is "pH"). Determine the buffer value for the solution that is a buffer. (Remember that slope is rise over run.)

3. Buffers resist change in pH when acid or base is added to them. Blood contains a number of buffers. Can you think of any ways in which the presence of such buffers in the blood would be of value to homeostasis?

LABORATORY ACTIVITY 18:

BUFFERING CAPACITY OF SOME BLOOD COMPONENTS

INTRODUCTION:

In the preceding activity you determined the effect of addition of acid or base on the pH of various solutions. You found that one of the solutions acted as a buffer: it resisted a pH change. In this activity you will be concerned with the buffering capacity of some blood components.

In Sections 10 to 17 we have discussed many of the components of blood. We have also indicated the importance of maintaining a constant pH in blood. The purpose of this activity is to investigate the degree to which some components of blood act as buffers and aid in the maintenance of the blood pH. The specific components to be tested are water, phosphates, sodium bicarbonate, sodium chloride and albumin (protein). To make the exercise more realistic, these components will be tested at concentrations close to those present in normal blood plasma. Also the pH of the solutions will have been adjusted to about 7.4 (the normal pH of arterial blood).

The procedure used in this investigation differs from that used in Activity 17. In that activity, HCl was added to each solution with a medicine dropper, and pH paper was used to determine the pH of the solutions. In this activity, a buret will be used instead of a medicine dropper and the pH will be measured by a color indicator or a pH meter rather than pH paper. The indicator dye, Congo red, that is used in this activity changes color at a pH close to 4.

MATERIALS:

phosphate salt solution
sodium bicarbonate solution
albumin solution
sodium chloride solution
0.1 M HCl solution
Congo red indicator solution
special water solution
buret
beaker, 50-ml
4 Erlenmeyer flasks, 125-ml
graduated cylinder, 10-ml
graduated cylinder, 100-ml
ring stand and clamp
BIP pH meter
pH probe
graph paper

PROCEDURE:

1. Choose one of the following to titrate: phosphate salt solution, sodium chloride solution, sodium bicarbonate solution or water. Record your choice. Since you will be sharing a buret with other students, each person in your group must select a different solution to titrate in order to obtain a complete set of data.

2. Transfer 10 ± 0.1 ml of the solution you selected to a 125-ml flask and label the flask.

3. Add one drop of Congo red indicator to the flask.

4. Set up the buret in preparation for titration.

5. Record the initial volume of the 0.1 M HCl in the buret.

6. Add 0.1 M HCl a drop at a time with continuous swirling until you note a color change that persists. Record the final reading. (It may be helpful to place a sheet of white paper under the flask while titrating.)

7. Determine the volume of HCl added by subtracting the initial reading from the final reading. Record this value. (Note: If you overshot the endpoint, i.e., added too much HCl, repeat the procedure.)

8. Check the results of the first run by making a second run. Again, record the initial and final volume, and the volume of HCl titrated.

9. The volumes in the two runs should not differ by more than 0.1 ml. If your two results are in this range, record the average volume and proceed to Step 11.

10. If the volumes in the two runs differ by more than 0.1 ml, make a third run. Record the results of this run and the average volume titrated for all three runs.

11. Share your data with the other members of your group.

12. Sometime during the laboratory period, you will have a chance to use the pH meter. To avoid a bottleneck, proceed immediately to the pH meter when it is available for your group. When you do this, you will titrate an albumin solution with 0.1 M HCl as in Steps 2 through 7, but the procedure will differ in some ways as explained below.

13. Use a graduated cylinder to add 20 ± 1 ml of albumin solution to a 50-ml beaker.

14. Rinse the pH probe and then dip it into the albumin solution and allow it to sit for 60 seconds.

15. Turn the mA dial on the BIP until the diodes null and record the pH. ($pH = \frac{mA\ reading}{10}$) (Note: Don't be concerned if the initial pH is not exactly 7.4. The pH of each solution was adjusted to approximately 7.4 by your instructor. If the pH was not 7.4 ± 0.2, then 0.1 M HCl or 0.1 M NaOH was added to bring the pH within one drop of 7.4.)

16. Record the level of the meniscus in the buret. Remove the probe from the solution and add 40 drops of 0.1 M HCl to the beaker. (Be careful when moving the probe--it is delicate.) Swirl to mix and return the pH probe to the solution. Wait 60 seconds before checking and recording the new pH. (See following Sample Data Table.)

SAMPLE DATA SHEET:

Drops 0.1 M HCl added	buret reading (ml)	Volume of 0.1 M HCl added (ml)	ph of albumin solution
0	24.5	0	7.4
40	26.3	1.8	6.4

17. Repeat Step 16 four more times.

18. Continue titrating but from now on add only 20 drops of HCl each time. When you reach a pH of 4.0, stop and record the final level of the meniscus. Rinse off the probe and put it back in the beaker of water near the pH meter.

19. Notify your teacher immediately after you are finished with the pH meter so that another group can get started.

20. Calculate the volumes of 0.1 M HCl used for titrating the albumin solution.

21. Graph the data collected for the albumin plotting "pH" on the x-axis and "ml 0.1 M HCl added" on the y-axis. Draw a "best" straight line through the plotted points. For a comparison, plot the data for the other solutions titrated on the same graph. For these solutions, assume that the initial pH was 7.4. Also assume that the final pH was 4.0, the approximate end-point for a Congo red titration. (You will have only these two points to plot.) Be sure to label each line. Determine the buffer value for each of the solutions.

DISCUSSION QUESTIONS:

1. List the solutions in terms of decreasing buffer strength. This list indicates which are the most important buffers in blood. For example, your data should show that the phosphate salt solution is not a good blood buffer. This fact is of some interest because it is also known that phosphate salts are important buffers in cells. Speculate on how a buffer can be ineffective in blood and yet effective in cells.

2. Can you think of any other substance(s) in plasma that might have a buffering effect?

3. Hemoglobin is one of the most important buffers of blood. Why is it unsuitable to test in the Congo red titration procedure used in this activity?

4. In Activity 15, you titrated a simulated antigen solution against a simulated antibody solution. How does the "titration" in that activity differ from the one in this activity?

5. Compare the techniques used in this activity and the preceding activity with

regard to precision. Which method do you think was most precise? Why? (The pH meter reaches to ± 0.1 pH unit.)

6. If you were asked to do an error analysis for this activity, what factors would you take into consideration? (Hint: What factors were involved in the error analysis in the vitamin C activity in the Nutrition Unit?)

ACTIVITY 19:

DETERMINING THE EFFECT OF DIET ON URINARY OUTPUT

INTRODUCTION:

This activity and Laboratory Activity 20 are a three-day sequence which will demonstrate how various changes in the diet can alter the composition of urine. Basically, you will be analyzing your urine for five characteristics.

1. urea concentration
2. pH
3. chloride ion concentration
4. calcium ion concentration
5. specific gravity

The first analysis will be made on a sample of normal urine, collected subsequent to following your normal diet. In the meantime, you will be asked to choose and follow, for one day, a special diet. A second urine specimen will then be analyzed. At the conclusion of the sequence, you will compare the results of the two analyses to determine the effects of the change in your diet.

The following is an outline of the complete activity on a day-by-day basis.

Day 1:

Plan your special 24-hour diet (in class).
Take home a specimen container.
Start your special diet with dinner.
Take a timed urine sample, from immediately after dinner until bedtime. This is your normal or "pre-diet" urine sample. The special diet won't affect the urine this quickly.

Day 2:

Remain on special diet all day, through dinner.
Take your pre-diet urine specimen to school.
Analyze pre-diet urine.
Take home a specimen container.
Take a timed urine sample, from immediately after dinner until bedtime. This is your "post-diet" urine sample.

Day 3:

Take your post-diet urine specimen to school.
Analyze the post-diet urine, and compare the two sets of results.

PART I: SELECTING YOUR ABNORMAL DIET

The components of your diet which may be altered include protein, salt and calcium. These substances have been selected to coincide with the tests to be performed on the urine samples. You have the option of increasing or decreasing your intake of any or all of these substances. The following guidelines may help you in designing your abnormal diet.

1. Protein: With the aid of USDA Home and Garden Bulletin 72, simply reduce the amount of protein in your diet. For example, eliminate meat and dairy products and select vegetables that are low or lacking in protein. (One day on a low-protein diet will not cause any person harm.) An alternative is to increase the amount of dietary protein. In this case, you may also wish to decrease carbohydrates in order to stay within your normal caloric intake.

2. Salt: For a low-salt diet, use Food Values of Portions Commonly Used by Bowes and Church to select foods that have little or no sodium. Note the kinds of foods that fall into the low-salt category. Be sure you don't add salt to any food; also avoid pre-prepared foods, since almost all of them contain salt (e.g., potato chips, lunch meats, canned vegetables). Also, cheese and most butter contain a significant amount of salt. You also have the option of increasing your salt intake, but doing this for more than 24 hours is not recommended. Recall that high levels of dietary sodium over long periods of time may be related to high blood pressure and cardiovascular diseases.) In the laboratory, the test for chloride ion concentration will be used as an indirect measure of sodium chloride concentration.

3. Calcium: Check USDA Home and Garden Bulletin 72 to see which foods are high or low in calcium. Increasing your calcium intake can be done by simply drinking more milk. Non-fat milk can be used to stay within your normal caloric intake on a high-milk diet. For a low calcium diet, simply avoid all dairy products.

Finally, it is suggested that your fluid intake be kept reasonably constant. Consumption of large amounts of water can produce a dilute urine which may reduce the accuracy of your test results, Avoid diuretics such as coffee, tea and alcohol.

Record the nature of the diet you select (i.e., high-protein, low-salt, etc.). Keep notes on foods eaten or not eaten that represent departures from your normal diet. Also record any special observations during the period of the abnormal diet (e.g., thirsty, hungry, change in frequency of urination, etc.)

PART II: COLLECTING A TIMED URINE SAMPLE

A timed urine sample refers to a measured volume of urine taken over a known period of time. Immediately after dinner, urinate and record the time. Do not collect this specimen. The purpose of this first urination is to provide a starting time with the bladder empty.

From this point on, collect all urine until bedtime. Record the time each time you urinate and the total volume of urine collected. The volume can be read directly off the specimen container.

Special note: If it appears that you may exceed the capacity of the specimen

container, some extra steps will be necessary. At some point, record the volume of urine collected thus far, and transfer the urine to a larger jar which may be discarded later. At the end of the collecting period, record the volume of urine now in the specimen container. Add the two volumes and record the sum. Add the urine in the specimen container to the jar and swirl to mix. Return at least 100 ml of urine to the specimen container and discard the remainder. The reason for mixing is that the composition of urine is constantly changing. By mixing the entire sample collected over the time period, you should obtain a good representative sample.

From the timed sample, you can estimate the volume of urine excreted in a 24-hour period. For example, if you collect 225 ml over a period of 200 minutes, your 24-hour total will be

$$\frac{225 \text{ ml}}{200 \text{ min}} \cdot \frac{60 \text{ min}}{\text{hr}} \cdot \frac{24 \text{ hr}}{\text{day}} = 1620 \text{ ml/day}$$

This information will also help you calculate the quantity of calcium, urea and chloride excreted per day. For example, if tests indicate that your urine contains 500 mg chloride per 100 ml, then your daily production would be

$$\frac{500 \text{ mg}}{100 \text{ ml}} \cdot \frac{1620 \text{ ml}}{\text{day}} = 8100 \text{ mg/day} = 8.1 \text{ g/day}$$

Using the data you have collected, calculate your 24-hour total volume as done in the preceding example. Record this figure. Remember to save all the data and your calculations. Remember to take your urine sample to school in the morning.

Preparing for Laboratory Activity 20:

Successful completion of Laboratory Activity 20 will require some teamwork among members of each group. It is advisable to read through each procedure carefully before class begins so that you can make the most of your lab time. In some cases, the exact equipment requirements have not been specified. It is expected that by now you will be able to decide what equipment is best suited for a particular task. A sample data sheet has been included at the end of Activity 20 to give you an example of how to organize your results.

None of the procedures is particularly difficult. However, unless you are careful and precise with your lab technique, you will not get meaningful results. For instance, always use clean pipets, and do not transfer pipets from one solution to another. Instead, keep one pipet in each solution.

LABORATORY ACTIVITY 20:

TESTING URINE SAMPLES RESULTING FROM NORMAL AND SPECIAL DIETS

GENERAL INTRODUCTION:

This activity consists of five different procedures for the five tests to be made on your urine samples. Two members of your group should complete the tests for urea and pH, while the other two determine the chloride ion and calcium ion

concentrations and specific gravity. Each test should be performed by the same pair on all four urine samples.

The procedures should be repeated on the second day for the "post-diet" specimens. But this time, reverse roles so that everyone will have a chance to perform all five tests over the two-day period.

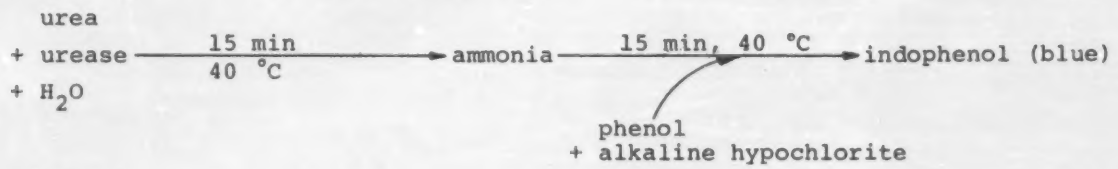
PART I: TEST FOR UREA CONCENTRATION

INTRODUCTION:

When protein is digested, it is broken down into amino acids and absorbed into the bloodstream. The amino acids can then be utilized to make more protein, depending upon the body's needs. When excess amino acids accumulate in the bloodstream, the nitrogen portion of these amino acids is converted in the liver by a multi-step metabolic process into urea, which can then be excreted by the kidneys.

Determination of blood-urea levels is currently one of the most common tests for kidney failure. Urea concentration will begin to increase if 70% of kidney function is lost, but changes in blood pH, Na^+, K^+ and other substances do not appear until approximately 90% of kidney function is lost.

In this test, the enzyme urease converts urea into ammonia, which then reacts with phenol and alkaline hypochlorite to form indophenol. You can measure the % transmittance of the blue color of the indophenol using the BIP colorimeter.



The unknown is compared to a standard graph which is made from urea solutions of known concentration. One note of caution: the accuracy of this procedure depends largely upon careful and precise pipetting technique. It is important to read the procedure over thoroughly before beginning.

MATERIALS:

- urea standard solution (10 mg/100 ml)
- urease solution
- alkaline hypochlorite solution
- phenol color reagent
- water bath, 40 °C
- thermometer, 0-100 °C
- 12 test tubes, 16 x 125 mm, Pyrex
- 15 pipets, 1-ml (or fewer with rinsing)
- 3 pipets, 10-ml
- graduated cylinder, 100-ml
- test-tube rack
- BIP colorimeter
- 8 cork stoppers, #4
- 4 urine specimens
- graph paper

PROCEDURE:

1. Program the BIP for colorimetry and plug it in.

2. First it will be necessary to dilute the urine specimens in order to bring the urea concentrations into the range where the colorimeter calibration curve is most nearly linear. Do this by placing 1 ± .01 ml of urine in a 100-ml graduated

cylinder. Dilute to 100 ± 1 ml with distilled water and mix thoroughly. Then place 1 ± .01 ml of this solution in a test tube, and add 9 ± .1 ml of distilled water. The urine has now been diluted by a factor of 1000. Label the test tube with the donor's initials.

3. Repeat Step 2 for the other three urine specimens.

4. Transfer 1 ± .01 ml of one of the diluted urine solutions into a clean cuvet. Label with the donor's initials. Repeat this step for the other three diluted urine solutions.

5. Number four cuvets from "1" to "4". These cuvets will contain dilutions of the urea standard solution. Make the additions listed in the table.

Cuvet Number	Volume Urea Standard Solution (ml)	Volume H_2O (ml)	Urea Concentration (mg/100 ml)
1	0	1.0 ± .01	0
2	0.2 ± .01	0.8 ± .01	2.0
3	0.4 ± .01	0.6 ± .01	4.0
4	0.6 ± .01	0.4 ± .01	6.0

6. You should now have eight cuvets, each containing 1 ml of solution: four with known urea concentrations and four with unknown urea concentrations.

7. Add 1 ± .01 ml of urease solution to each tube and mix.

8. Incubate all tubes at 40 °C for 15 minutes. Note: the exact temperature is not critical. A container of warm water will be adequate, if kept near 40 °C.

9. In this step two reagents are added to the tubes. It is important to work quickly and to mix thoroughly after the addition of each reagent. First add 2 ± .1 ml of the phenol color reagent stopper, and mix, then add 2 ± .1 ml of the alkaline hypochlorite solution, re-stopper and mix.

10. Incubate all tubes at 40 °C. for 15 minutes.

11. Add 5 ± .1 ml distilled water to each tube and swirl to mix.

12. Standardize the colorimeter, using cuvet 1 as the blank.

13. Determine and record the % transmittance of each solution. Convert the values to absorbances and record.

14. Prepare a calibration line by plotting the known urea concentrations versus absorbance. Then determine and record the unknown concentrations from the graph.

15. Since the urine samples were diluted by a factor of 1000, it will be necessary to multiply each result obtained in Step 14 by 1000. Record the urea

concentrations of the undiluted samples (in mg/100 ml).

16. Convert your results from mg/100 ml to mg/day. To do this, you will need to obtain the 24-hour total urine volume from each member of your group. Share your results with the other members of your group.

PART II: pH DETERMINATION

INTRODUCTION:

Urine pH can fluctuate due to changes in diet and general state of health. Large amounts of fats in the diet can produce a more acid urine. Bacterial infections of the bladder can alter the urine pH in either direction, depending upon the waste products of the bacteria.

MATERIALS:

5 beakers, 50-ml
BIP pH meter with pH electrode (1 per class)
4 urine samples

PROCEDURE:

1. Place approximately 20 ml of each urine sample to be tested in separate beakers. Label the beakers with the appropriate initials.

2. When the pH meter is available to you, use it to determine the pH of each sample.

3. Remember to wait at least 30 seconds before turning the mA dial to the null setting and reading the pH. Also, be sure to rinse the probe in distilled water between readings. Record the readings and share your results with the other members of your team.

PART III: TEST FOR CHLORIDE ION CONCENTRATION

INTRODUCTION:

Chloride ion is tested for as an indirect measure of NaCl concentration in the urine. The unknown urine sample is titrated with a mercury II nitrate solution using diphenylcarbazone as an indicator. The mercury ions combine with chloride to form mercury II chloride.

$$Hg(NO_3)_2 \longrightarrow Hg^{++} + 2\ (NO_3)^-$$

$$2\ Cl^- + Hg^{++} \longrightarrow HgCl_2$$

After all chloride ions have reacted, excess Hg^{++} combines with diphenylcarbazone to form a blue-violet colored substance, which marks the end of the titration.

MATERIALS:

mercury II nitrate solution,.0141 M
diphenylcarbazone indicator solution
buret, 50-ml
beaker, 50-ml
2 pipets, 1-ml
pipet, 10-ml
ring stand and clamp
4 urine samples

PROCEDURE:

1. Fill a buret with $Hg(NO_3)_2$ solution.
2. Place 1 ± .01 ml of the urine sample to be tested in a 50-ml beaker.
3. Add 0.3 ± .05 ml diphenylcarbazone indicator and 5.0 ± .1 ml distilled water to the beaker.
4. Record the buret reading at the beginning of the titration. Titrate a drop at a time with constant mixing until a slight blue-violet color persists. Record the buret reading when the endpoint is reached.
5. Repeat Steps 2 through 4 for the remaining urine samples.
6. Calculate and record the volumes of $Hg(NO_3)_2$ solution needed to complete the titrations.
7. Calculate the chloride ion concentration in mg/100 ml for each sample.

Note: the concentration of the mercury II nitrate solution has been chosen so that 1 ml of $Hg(NO_3)_2$ solution will combine with 1 mg of chloride ion. To calculate the chloride concentration in mg/100 ml, simply multiply the volume of $Hg(NO_3)_2$ solution used by 100. Thus, if 3.1 ml are needed to complete the titration, the chloride concentration is found as follows.

$$(3.1 \text{ ml } Hg(NO_3)_2 \text{ solution}) \cdot \frac{1 \text{ mg } Cl^-}{\text{ml } Hg(NO_3)_2 \text{ solution}} = 3.1 \text{ mg } Cl^-/\text{ml urine}$$

$$= 310 \text{ mg } Cl^-/100 \text{ ml urine}$$

8. Convert mg/100 ml to mg/day. Share your results.

PART IV: TEST FOR CALCIUM CONCENTRATION

INTRODUCTION:

In this test, urine is mixed with an equal volume of Sulkowitch reagent. This reagent contains oxalate ions which, when added to urine, produce a fine, white precipitate of calcium oxalate. The resulting turbidity can be compared with standard solutions of calcium using the BIP colorimeter.

MATERIALS:

- Sulkowitch reagent
- calcium standard solution, 50 mg/100 ml
- 8 cuvets, 16 x 125 mm
- test-tube rack
- 7 pipets, 10-ml (or fewer with rinsing)
- BIP colorimeter
- 4 urine specimens
- graph paper

PROCEDURE:

1. Program the BIP for colorimetry and plug it in.
2. Number four cuvets, from "1" through "4". Make the additions listed in the table on the following page.

Cuvet Number	Ca^{++} Standard Solution (ml)	H_2O (ml)	Calcium Concentration (mg/100 ml)
1	0	5 ± .1	0
2	1 ± .1	4 ± .1	10
3	2 ± .1	3 ± .1	20
4	3 ± .1	2 ± .1	30

3. Add 5 ± .1 ml of one of the urine specimens to a clean cuvet. Label with the donor's initials. Repeat this step for the other three urine specimens.

4. You should now have eight cuvets, each containing 5 ml of solution: four with known calcium concentrations and four with unknown calcium concentrations.

5. Add 5 ± .1 ml of Sulkowitch reagent to each tube. Mix and allow to stand for 2 to 3 minutes.

6. Standardize the colorimeter, using Cuvet 1 as the blank.

7. Determine and record the % transmittance of each solution. Convert the values to absorbances and record.

8. Prepare a calibration line by plotting the known calcium concentrations versus absorbance. Then determine and record the unknown concentrations from the graph.

9. Convert your results from mg/100 ml to mg/day. Share your results.

PART V: SPECIFIC GRAVITY

INTRODUCTION:

The determination of specific gravity is an important part of routine urinalysis and is widely used because of its speed and simplicity. Specific gravity is the ratio of the mass of a substance to the mass of an equal volume of water, and is related to the masses of compounds other than H_2O in the urine. Although the specific gravity of urine may fluctuate markedly during the course of a day, the procedure does have value as a screening test in checking the concentrating ability of the kidney tubules. This function is generally the first to be lost when the kidney tubules are damaged.

MATERIALS:

urinometer float and cylinder
4 urine specimens
graduated cylinder, 100-ml

PROCEDURE:

1. Pour 15 ± 1 ml of a urine sample into the cylinder of the urinometer.

2. Place the urinometer float in the cylinder with a slight twisting motion

so that it does not stick to the cylinder wall.

3. Read the scale where the meniscus crosses the stem of the float. Examining the stem of the float before you start may help you understand how to read the markings.

4. Record the specific gravity reading and repeat for the other samples. Share your data.

REMEMBER TO COLLECT A TIMED URINE SPECIMEN TONIGHT FOR ANALYSIS OF THE "POST-DIET" URINE.

LABORATORY ACTIVITY 21:

THE ARTIFICIAL KIDNEY MODEL

INTRODUCTION:

This is the beginning of a four-day sequence in which you will design a model which will function, in principle, like an artificial kidney. Most previous activities have provided a set of specific procedure steps. In this activity you will be given certain resources and information as you ask for it, but it will be up to you to plan a model which you can then construct and finally test. Keep notes on all your work. A cooperative effort with other students will be needed because there will not be enough time for one student to do all of the work. As you complete one part of the activity, go on to the next. The sequence has been divided up merely to give you a working guide.

The Problem:

You will be provided with a 100-ml sample of simulated blood which contains chloride, bicarbonate and urea in the amounts present in the blood of someone with uremia. Uremia is a blood abnormality related to poorly functioning kidneys. The kidney condition is referred to as "kidney failure." When you have finished making the artificial kidney model, you will put this blood through it and then analyze the treated blood to see how well the apparatus works. The model is successful if the blood composition is changed to a composition more like that of normal blood. The following table provides typical concentrations for normal and uremic blood.

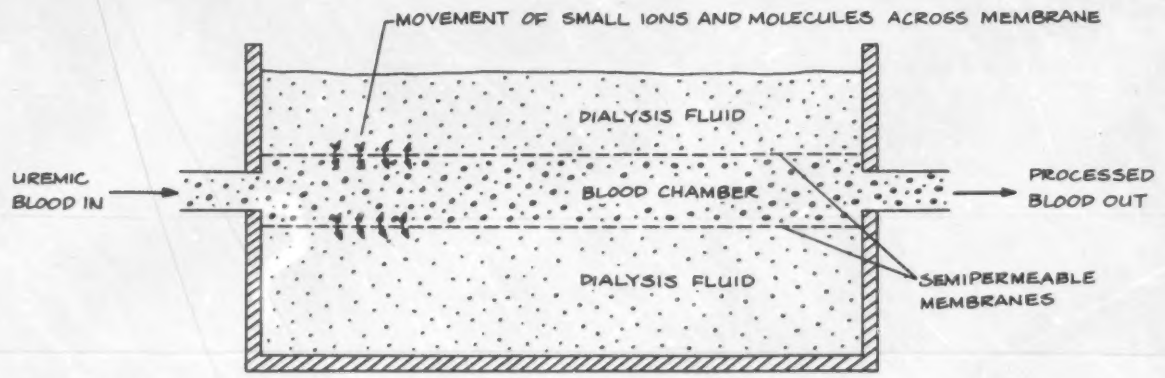
MATERIAL	NORMAL BLOOD (mg/100 ml)	UREMIC BLOOD (mg/100 ml)
Cl^-	380	415
HCO_3^-	165	85
Urea	25	200

The principle is to treat the uremic blood in such a way that $[Cl^-]$ is reduced slightly, $[HCO_3^-]$ is increased significantly and urea is removed as completely as possible.

The Solution:

The best approach is to use a process called "dialysis." In dialysis, two solutions are separated by a thin membrane which is permeable to small ions and molecules, but not to larger particles such as blood cells and proteins. Substances pass through

the membrane, moving from the side of higher concentration to that of lower concentration. In an artificial kidney, the patient's blood is kept on one side of the membrane, while a specially formulated "dialysis fluid" is kept on the other. (See figure.) By adjusting the relative concentrations of small ions and molecules, dialysis restores the uremic blood to its proper composition.



The Plan:

Work in groups of two or three. The activity will run for only four or five periods, so in order to get everything done, each person must do his or her share. Some of your time each day will be spent in testing the materials, constructing the artificial kidney model and consulting with the other members of your team.

The following is an outline of the entire activity. It will be up to you to:

1. formulate and prepare the dialysis fluid,
2. select the proper materials for the dialyzer,
3. design the artificial kidney model,
4. build the model,
5. test the model,
6. process the uremic blood and test it to see how well the artificial kidney model works.

PROCEDURE: (Periods 1 and 2)

The objectives are to:

1. formulate and prepare the dialysis fluid,
2. become familiar with the model materials,
3. select the dialysis membrane material,
4. begin planning the design of the artificial kidney model.

Preparation of the Dialysis Fluid:

Review the table comparing uremic blood and normal blood. Decide what the concentrations of Cl^-, HCO_3^- and urea should be in the dialyzing fluid. Should they be the same as in normal blood? When you have decided on a trial recipe for the dialysis fluid, show the recipe to your teacher. If approved, the mixture can be made

up and stored for use later. Make up at least one liter.

Selecting the Dialysis Membrane Material:

Several materials will be available for consideration and testing. These will include sheet plastics such as polyethylene and cellophane; some may also be available in tubing form. Not all will work well. You will have to test them to make a selection. Remember that you are searching for a membrane that will allow small molecules to pass through easily. Use whatever reagents and equipment are available to help you make your decision.

Becoming Familiar with the Materials:

The following is a partial list of materials, besides the plastic film, which you should consider in making your artificial kidney model.

- adhesives such as white glue, shellac
- sealants such as silicone rubber (this takes a day to cure completely)
- beakers, flasks, glass tubing, pipets, ring stands and clamps, flexible rubber and plastic tubing, rubber and cork stoppers, etc.
- rubber bands, string, heavy thread, masking tape
- tongue depressor blades, applicator sticks, cardboard, etc.
- you may also bring things from home, such as cardboard, plastic trays, and prepare parts of your artificial kidney model at home.

Designing the Artificial Kidney Model:

After you have chosen a dialyzing membrane, you should begin planning a design. At first keep it fairly simple; as you become more familiar with the materials, you may want to modify the design before you build the final version. Here are some things to keep in mind.

1. The amount of simulated blood will be small, about 100 ml. Consequently, the volume of the "kidney's" blood chamber should be small.

2. The amount of dialysis fluid should be large--at least 1000 ml.

3. The uremic blood is colored with a red dye; thus you will know if you have leaks or a bad system, because the color will get into the dialysis fluid.

4. The area of contact between blood and dialysis fluid is important; the greater the surface area of the membrane, the more effective the artificial kidney model will be.

5. Anything that comes in contact with the uremic blood or the dialyzing solution must be waterproof. This means that if you use cardboard, cloth or paper, it will have to be treated with shellac or coated with silicone rubber.

6. The amount of dialysis that takes place depends, to a large extent, on the number of particles that can touch the membrane. Therefore, some provisions should be made for circulating the fluids. Gravity, stirring and an aquarium pump are all possibilities.

7. Connections should be leak-free. For example, a connection between plastic tubing and dialysis tubing could be made by both silicone rubber and string.

8. Too great a pressure difference across the membrane can cause the plastic to break or collapse. The trial run will help identify any problem areas.

Keep a detailed record of the steps involved in the design of your artificial kidney model. The following information should be included: (1) the concentration of Cl^-, HCO_3^- and urea in the dialysis fluid, (2) the quantities of chemicals to be used to obtain these concentrations, (3) a list and description of the materials you will use, (4) a sketch of the model and (5) a short summary explaining how the model is expected to function. This record will require approval from your instructor before you begin to construct the apparatus. If there are serious flaws in the design, you will be given a Trouble Shooting Worksheet to help you redesign the model. If there are only minor problems, they will become apparent from your laboratory results and you will have an opportunity to make needed corrections.

GUIDELINES FOR USE OF MATERIALS:

WHITE GLUE (casein or other glue) serves as a strong adhesive for wood, paper or cardboard. It requires 24 hours for a complete set. Pressure is usually helpful. This glue can deteriorate if it gets wet, so it should not be exposed directly to liquids.

SHELLAC serves as a good waterproofing agent when spread over cardboard, cloth or paper, and takes at least 2 to 4 hours to dry. Several coats may be necessary. If brushes are used, clean them thoroughly with denatured alcohol after use. Even better, the materials may be allowed to soak in shellac for several minutes. Acrylic plastic spray dries more rapidly and is somewhat less messy than shellac. Several coats may be necessary.

SILICONE RUBBER (also called "silicone adhesive" or silicone sealant") also serves as a waterproofing and sealing agent, although it is difficult to apply in a thin coat. It can bind wood, glass, cardboard, fiberglass, and rubber, but will not stick to plastic films, especially when they are wet. Silicone rubber can be very useful. It takes 24 hours for a complete cure.

PLASTIC FILMS: The following table lists some relative characteristics of the plastic membranes available in the classroom.

	MATERIAL* (thickness typically 0.001 inches)			
	cellophane, uncoated	Mylar	polyethylene (Handi-wrap)	Saran-wrap
water absorption	75	4	0.01	0.0
water vapor transmission	20	1.7	1.0	0.3
gas permeability (O_2)	1	7	500	2

* The larger the number, the more permeable the plastic is(to water, water vapor or O_2). For example, polyethylene is very permeable to gases which explains why food wrapped tightly in polyethylene can still release odors inside your refrigerator.

LABORATORY ACTIVITY 22:

BUILDING THE ARTIFICIAL KIDNEY MODEL

PROCEDURE: (Periods 2 and 3)

The objectives are to:

1. complete the design,
2. construct the artificial kidney model,
3. run preliminary tests and
4. plan for the BIG DAY.

The Final Design and Construction of the Artificial Kidney Model:

Review the suggestions or comments given you by your instructor. When your design has been approved, you can begin construction. Depending on design, you may want to make small changes as you go along. For example, leaks may develop with certain designs and changes may be needed. Try to complete construction in one period so you can make some trial runs during the next period. The trials can test for leaks, determine flow rates, adjust for gravity effects, etc. You should also plan a trial test of a solution of your own making to be sure that dialysis is occurring.

Running Preliminary Tests:

At first, run the red dye to check for leaks. If no leaks are immediately apparent, run a test sample for 10 to 15 minutes to see whether the apparatus works at all. You can test the artificial kidney model in any way you wish. You may want to run a sample of bicarbonate solution. This would give you some experience in performing the bicarbonate test described in Laboratory Activity 23. Or you could run a sample of urea (200 mg/100 ml). After 10 to 15 minutes, the processed sample can be compared to the original sample using the test for urea described in Laboratory Activity 23. If there is no detectable difference between the processed sample and the original sample, you can be almost sure that the artificial kidney model will not work well enough on the uremic blood.

Planning for the BIG DAY:

In the final period, your teacher will give you 100 ml of uremic blood and you will put it through your artificial kidney model. The composition of the blood will be:

Cl^-	415 mg/100 ml
HCO_3^-	85 mg/100 ml
Urea	200 mg/100 ml
Protein	2.0 g/100 ml, stained red

You should process the uremic blood for 30 minutes. During the processing, one team member may run brief checks to see whether dialysis is occurring (for example, a pH check might be used). Such tasks should be planned a day in advance so that there is no time wasted on the last day. You should also be making preparations for the tests on chloride, bicarbonate and urea. Decide which member of the group will do each job. Review the material in Laboratory Activity 20 and the new procedures in Laboratory Activity 23.

LABORATORY ACTIVITY 23:

THE BIG DAY

GENERAL INTRODUCTION:

Objectives:

1. to process 100 ml of uremic blood for 30 minutes;

2. to analyze the blood for Cl^-, HCO_3^- and urea to see how well the artificial kidney model works.

Your team will get 100 ml of uremic blood to process in the artificial kidney model. Avoid losing any of the blood. Make frequent checks to determine whether the apparatus is functioning properly (i.e., does not develop leaks, etc.). While the blood is being processed, preparations should be made for the analyses.

Analysis for Cl^-, HCO_3^- and urea:

Following the processing, your team will analyze the processed blood for changes in the concentrations of Cl^-, HCO_3^- and urea. Divide up the tests so that there is enough time to complete them all. It is advisable to read through these tests carefully in advance. The test for bicarbonate is new to you, while the tests for urea and chloride are modified versions of those used in Laboratory Activity 20.

PART I: TEST FOR UREA

INTRODUCTION:

The test for urea is virtually identical to the analysis carried out in Activity 20. Both the uremic blood and the processed blood are initially diluted by a factor of 20. The diluted uremic blood is then used as the standard (10 mg urea/100 ml) to which the processed blood is compared. It may be desirable to collaborate with other teams since the standard curves can be shared, as long as the same BIP and test well are used by both groups.

One other point of information: In Laboratory Activity 20, the unknowns were diluted by a factor of 1000, and 1 ml of this solution was used as the sample volume. The urea levels were calculated by simply multiplying the resulting concentrations by 1000. In this procedure, it is necessary to test several different dilutions in order to be sure that one or two of them fall within a range that can be measured properly by the BIP colorimeter. In order to calculate the concentrations of your unknowns, it is necessary to multiply by a different dilution factor for each sample. These factors are listed on the right side of the table shown in Step 3. They were calculated by taking the reciprocals of the dilutions made on each sample. For instance, in cuvet 5, 0.4 ml of the processed blood is diluted to a final volume of 1 ml (the blood had already been diluted by a factor of 20). The dilution factor is:

$$\left(\frac{1}{\frac{1}{20}}\right) \cdot \left(\frac{1}{\frac{4}{10}}\right) = 50$$

MATERIALS:

uremic blood (to be made into a urea standard solution

urease solution

alkaline hypochlorite solution

phenol color reagent
water bath, 40 °C
10 cuvets, 16 x 125 mm, Pyrex
6 pipets, 1-ml
3 pipets, 10-ml
test-tube rack
BIP colorimeter
cork stoppers, #4
graph paper

PROCEDURE:

1. Program the BIP for colorimetry and plug it in.

2. Dilute the uremic blood and the processed blood by a factor of 20. This can be done by placing 0.5 ml of the blood in a test tube and adding 9.5 ml of distilled water.

3. Number eight cuvets. Make the dilutions listed in the following table.

Cuvet No.	Volume diluted uremic blood (ml)	Volume H_2O (ml)	Concentration (mg/100 ml)	
1	0	1.0 ± .01	0	
2	0.2 ± .01	0.8 ± .01	2	
3	0.4 ± .01	0.6 ± .01	4	
4	0.6 ± .01	0.4 ± .01	6	
	Volume diluted processed blood (ml)			Dilution factors
5	0.4 ± .01	0.6 ± .01		50
6	0.6 ± .01	0.4 ± .01		33
7	0.8 ± .01	0.2 ± .01		25
8	1.0 ± .01	0		20

4. Add 1 ± .01 ml of urease solution to each cuvet and mix.

5. Incubate all cuvets at 40 °C for 15 minutes. Note: the exact temperature is not critical. A container of warm water will be adequate, provided that the water is kept near 40 °C.

6. Add and mix quickly, one after the other, 2 ± .1 ml of the phenol color reagent, and 2 ± .1 ml of the alkaline hypochlorite solution.

7. Incubate all cuvets at 40 °C for 15 minutes.

8. Add and record 5 ± .1 ml distilled water to each cuvet and swirl to mix.

9. Standardize the colorimeter, using cuvet 1 as the blank.

10. Determine and record the % transmittance of each solution. Convert the values to absorbances and record.

11. Prepare a calibration line by plotting the known urea concentrations versus absorbance. The concentrations of the diluted unknowns may be read directly from the graph.

12. Determine the concentration of each unknown sample with an absorbance in the range of 0.10 to 0.70. (The other samples lie outside the range of "best" linearity.)

13. To convert to the correct values, multiply your results by the corresponding dilution factors. Find the average of the values obtained. Record the urea concentration of your processed blood on a data sheet. (A sample data sheet is provided at the end of the activity.)

PART II: TEST FOR CHLORIDE

INTRODUCTION:

This procedure is essentially the same as that used in Laboratory Activity 20. However, since there is only a small change in the chloride level in uremia, a more accurate measurement must be made. A larger sample volume is needed, and thus, more $Hg(NO_3)_2$ solution than in Activity 20. The processed blood is compared to the uremic blood by titrating both solutions to the same end-point.

MATERIALS:

- mercury II nitrate solution (0.0141 M)
- diphenylcarbazone indicator solution
- buret, 50-ml
- 2 beakers, 150-ml
- 2 pipets, 10 ml
- graduated cylinder, 100-ml
- ring stand and clamp
- distilled water

PROCEDURE:

1. Fill the buret with $Hg(NO_3)_2$ solution.

2. Place 5 ± .1 ml of uremic blood in a 50-ml beaker.

3. Add 1.5 ± 0.1 ml diphenylcarbazone indicator and 25.0 ± 1 ml water to the beaker.

4. Record the buret reading at the beginning of the titration. Titrate drop by drop with constant mixing until a slight blue-violet color persists. Due to the red tinge of the blood, the end-point is not easy to see, so titrate carefully. The total volume of $Hg(NO_3)_2$ solution used should be about 20 ml. Calculate and record the volume of $Hg(NO_3)_2$ solution used to the nearest 0.1 ml. Do not discard the blue-violet solution.

5. Repeat Steps 1 through 4 using 5 ± .1 ml of the processed blood. Titrate until the color produced is the same as in the first beaker. Record the volume of $Hg(NO_3)_2$ solution used.

	ml $Hg(NO_3)_2$
uremic blood	V_{UB} = ?
processed blood	V_{PB} = ?

6. The amount of $Hg(NO_3)_2$ used in the titration is directly dependent on the amount of Cl^- in a 5-ml sample of blood. Consequently, the following proportional relationship gives a value for the Cl^- concentration in the processed blood.

$$\frac{Cl^- \text{ concentration}}{415 \text{ mg}/100 \text{ ml}} = \frac{V_{PB}}{V_{UB}}$$

$$\text{or:} \quad Cl^- \text{ concentration} = \frac{V_{PB}}{V_{UB}} \cdot 415 \text{ (mg/100 ml)}$$

(V_{PB} and V_{UB} stand for the volume of $Hg(NO_3)_2$ solution used in the titration for the processed and uremic blood, respectively.)

PART III: TEST FOR BICARBONATE

INTRODUCTION:

This test is similar to the test for calcium used earlier. In this case, a saturated barium hydroxide solution is added to a diluted unknown. The turbidity resulting from the precipitation of $BaCO_3$ can be measured using the colorimeter.

$$Ba(OH)_2 + NaHCO_3 \rightleftharpoons NaOH + H_2O + BaCO_3$$

MATERIALS:

barium hydroxide solution, saturated ($Ba(OH)_2$)
4 pipets, 10-ml
graduated cylinder, 10-ml
graduated cylinder, 100-ml
3 cork stoppers, #4
3 cuvets, 16 x 125 mm, Pyrex
BIP colorimeter
beakers or flasks
test-tube rack
graph paper

PROCEDURE:

1. Dilute the uremic blood and processed blood each by a factor of 10.
2. Number three cuvets and make the additions listed in the following table.

Cuvet Number	Diluted uremic blood (ml)	$Ba(OH)_2$ (ml)	H_2O (ml)	Concentration (mg/100 ml)
1	5.0 ± .1	0	5.0 ± .1	0
2	5.0 ± .1	5.0 ± .1	0	8.5
3	Diluted processed blood (ml) 5.0 ± .1	5.0 ± .1	0	

3. Standardize the colorimeter, using Cuvet 1 as the blank.
4. Determine and record the % transmittance for Cuvets 2 and 3. They should be stoppered and inverted several times just before taking the reading so that the precipitate is well mixed. Convert % T to absorbance and record.
5. Prepare a calibration line by plotting the known concentrations (Cuvets 1 and 2) versus absorbance. Then determine and record the unknown concentration (Cuvet 3) from the graph.
6. Multiply the result by 10 to obtain the correct bicarbonate concentration.

DISCUSSION QUESTIONS:

1. In what ways does your processed blood compare favorably with normal blood? Unfavorably?

2. How would you modify your artificial kidney model in order to make it operate more efficiently?

3. If your artificial kidney model only partially restored the blood to normal, how long do you think it would take to complete the process?

4. In the test for bicarbonate, Cuvet 1 was used as a blank, even though it actually had just as much HCO_3^- as Cuvet 2. Do you think this was justified? Why?

SAMPLE DATA SHEET: (for final results only)

Substance	Uremic blood	Processed blood	Normal blood
Cl^-	415 mg/100 ml	_____mg/100 ml	380 mg/100 ml
HCO_3^-	85 mg/100 ml	_____mg/100 ml	165 mg/100 ml
Urea	200 mg/100 ml	_____mg/100 ml	25 mg/100 ml

Length of run: _____ min.

LABORATORY ACTIVITY X:

PREPARING ACID SOLUTIONS OF A GIVEN pH

GENERAL INTRODUCTION:

In Section 15 of the Mathematics Text, the following type of problem was considered.

> If you wished to make up a solution of acetic acid with a pH of 4.0, what initial concentration of acetic acid would you use? ($K = 1.8 \times 10^{-5}$).

It is often necessary to solve such problems, particularly when dealing with biological systems in a laboratory. This is because many biochemical substances (especially enzymes) function only within specific pH ranges. For example, in Unit II, in the nutrient analysis laboratory activity, the pepsin used to digest proteins was dissolved in an acid solution of approximately pH 1. This provided the acidic environment necessary for pepsin activity. (If the pH of that solution had been 8, very little or no digestion would have taken place.)

The purpose of this activity is to enable you to apply the calculations that you have been performing in Mathematics. In other words, can you solve the problem presented above, then use the results you obtain to make up a solution of acetic acid in the laboratory, and end up with a pH 4 solution? One way to answer this question is to go through the actual process of making up an acidic solution, using the results of your calculations, and then see how close you are to the desired pH. This is what you will do in this activity.

This activity is based on Problem 7 of Mathematics Problem Set 15. You will review the mathematical calculations and determine the initial concentration of benzoic acid needed to make a pH 4.2 solution. Then you will prepare a solution based on this initial concentration and use the pH meter to check the pH.

PART I: STATEMENT OF PROBLEM AND CALCULATIONS

PROBLEM: What initial concentration of benzoic acid is required to achieve an equilibrium pH of 4.2?

$$K = 6.3 \times 10^{-5}$$

$$C_6H_5COOH \rightleftharpoons H^+ + C_6H_5COO^-$$

CALCULATIONS:

1. Determine the hydrogen ion concentration, $[H^+]$, that corresponds to a pH of 4.2. Remember $[H^+] = 10^{-pH}$. In this case, $[H^+] = 10^{-4.2}$ which may also be written as $(10^{-5} \times 10^{.8})$. To determine the value of $10^{.8}$, make use of the following table. (Note: If you have already performed the calculations outlined in this and the following steps, then simply record your results for each calculation required.)

$10^0 = 1$	$10^{.3} \approx 2.0$	$10^{.6} \approx 4.0$	$10^{.9} \approx 7.9$
$10^{.1} \approx 1.25$	$10^{.4} \approx 2.5$	$10^{.7} \approx 5.0$	$10^{1.0} \approx 10$
$10^{.2} \approx 1.6$	$10^{.5} \approx 3.2$	$10^{.8} \approx 6.3$	

2. Complete the following table, on a separate piece of paper. If you are stumped, review Mathematics Section 15.

		$[H^+]$	$[C_6H_5COO^-]$	$[C_6H_5COOH]$
Concentrations [M]	Initial			
	Equilibrium			

3. Using the information recorded in the table, determine the concentration of undissociated benzoic acid, $[C_6H_5COOH]$, at equilibrium, (Remember,

$$K = \frac{[H^+][C_6H_5COO^-]}{[C_6H_5COOH]}$$

where all the concentrations are those at equilibrium.)

4. Determine the initial concentration of benzoic acid necessary to produce the equilibrium concentrations with which you are working. (Recall that the initial concentration of acid is equal to the sum of the concentrations of hydrogen ion and undissociated benzoic acid at equilibrium.)

5. Calculate the number of grams of benzoic acid per liter that corresponds to the initial concentration of acid you calculated in Step 4. (The gram molecular weight of benzoic acid is 122 g.)

6. Proceed to Part II, and make up your test solution based on the calculated concentration of benzoic acid (Step 5).

PART II: PREPARING THE TEST SOLUTION

INTRODUCTION:

The initial concentration of benzoic acid (calculated in Part I) requires a mass of benzoic acid too small to be measured out on the balances available to you. Therefore you will (1) make up a solution with a concentration of acid 100 times the desired concentration, and (2) dilute this solution 100-fold to give you your test solution. Note that by combining these two steps you will end up with a solution at the desired concentration. To prepare the benzoic acid solution in this manner, you will go through a series of steps similar to those outlined in the following example.

SAMPLE OPERATION:

Suppose the desired concentration of benzoic acid = .023 g/liter. You could make up a solution 100 times as concentrated as the desired concentration.

$$\frac{.023\ g}{liter} \times 100 = 2.3\ \frac{g}{liter}$$

But this would require a liter of distilled water and that would be wasteful. So instead you will weigh out only ten times as much benzoic acid (in our example, .23 g

rather than 2.3 g) and dissolve in one-tenth as much water (i.e., 100 ml rather than 1000 ml). Note that a concentration of $\frac{2.3\ g}{liter}$ is the same as $\frac{.23\ g}{100\ ml}$.

The resulting solution is 100 times the desired concentration (i.e., .023 g/liter), so dilute by 100-fold. Dilute 1 ml to 100 ml.

One final note before you begin. The water that you use to make up the solutions has been adjusted to pH 7.0. Therefore, use only this "prepared" water.

MATERIALS:

- benzoic acid (C_6H_5COOH)
- 2 Erlenmeyer flasks, 125-ml
- graduated cylinder, 100-ml
- pipet, 1-ml
- balance
- BIP
- pH probe
- stirring rod
- spatula
- "prepared" water (pH 7.0)
- gas burner
- ring stand with clamp
- wire gauze

PROCEDURE:

1. Note the mass of benzoic acid per liter calculated in Part I, Step 5. (Hint: The calculated value should be in the range of 0.01 to 0.02 g per liter. If your answer is not in this range, recheck your calculations.) Weigh out 10 times the calculated mass of benzoic acid.

2. Add to the acid enough "prepared" water to make 100 ml (not a liter) of solution. It is necessary to heat the solution to dissolve all the benzoic acid. Be sure all the acid is dissolved before proceeding to the next step.

3. Using the 1-ml pipet, transfer 1.0 ± 0.01 ml of acid solution to a clean 125-ml flask, and dilute to 100 ml with prepared water.

4. When there is an opportunity, proceed to the pH meter to determine the pH of your solution. Make sure that the pH probe has been carefully rinsed with distilled water before you insert it into your solution. Also remember to let the probe sit in the solution for 60 seconds before taking a reading. Record the pH of your test solution.

DISCUSSION QUESTIONS:

1. How close was the pH of your solution to that given in the problem?

2. Can you account for any difference between the measured and calculated pH?

3. In this activity you prepared a benzoic acid solution of pH 4.2. Can you think of another way to prepare a benzoic acid solution of this pH that does not involve performing the mathematical calculations?

LABORATORY ACTIVITY Y:

APPLICATION OF QUADRATIC EQUATIONS TO pH PROBLEMS

GENERAL INTRODUCTION:

In Laboratory Activity X you determined the initial concentration of benzoic acid necessary to make up a solution of pH 4.2. Then you made up the solution and checked the pH with a pH meter. This activity is similar; you will be finding the mathematical solution to a "pH problem" and then testing the results in the laboratory. However, this time you will be given the initial concentration of an acid and asked to calculate the pH of the solution that will result.

In Part I of the activity, you will perform calculations necessary to solve a pH problem. In Part II, you will prepare a solution based on the initial concentration given in the problem and check the pH to see whether it is the same as the pH you calculated.

The acid you will be using is ascorbic acid, vitamin C. The molecular formula is $C_6H_8O_6$. However, for convenience we will use the designation of "H_2Asc" for ascorbic acid and "$HAsc^-$" for the anion formed after the acid dissociates.

PART I: STATEMENT OF THE PROBLEM AND CALCULATIONS

PROBLEM: An ascorbic acid solution is made up with an initial concentration of 1×10^{-4} M. What is the pH of the solution that results?

$$H_2Asc \rightleftharpoons H^+ + HAsc^-$$

$$K = 8 \times 10^{-5}$$

CALCULATIONS:

1. Once you determine the hydrogen ion concentration $[H^+]$ at equilibrium, you can calculate the pH of the solution. In order to determine $[H^+]$ at equilibrium, first set the equilibrium $[H^+] = z$, and then complete the following table.

		$[H^+]$	$[HAsc^-]$	$[H_2Asc]$
Concentrations [M]	Initial	0	0	1×10^{-4}
	Equilibrium	z	z	?

2. Next substitute the expressions from the table for $[H^+]$, $[HAsc^-]$ and $[H_2Asc]$ at equilibrium, and K, into the equation.

$$K = \frac{[H^+][HAsc^-]}{[H_2Asc]}$$

3. Perform the operations necessary to arrive at a quadratic equation of the form

$$az^2 + bz + c = 0$$

(If you do not remember how to do this, refer to the example in Section 16 of the Mathematics Text.)

4. Once you have determined a, b and c, substitute their values into the following equation and solve for z. Use the square root table at the end of Unit III of the Mathematics Text.

$$z = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

(Remember that there will be two solutions for z, one negative and one positive. However, since z = $[H^+]$, and a negative value for $[H^+]$ has no meaning, you will be interested only in the positive solution to the equation.)

5. Now that you have a value for $[H^+]$ at equilibrium, you can calculate the pH of the solution. To do this follow the steps set forth in the example given below.

EXAMPLE: Suppose the $[H^+]$ you calculated was 7.9×10^{-5} M. We know that $[H^+] = 10^{-pH}$. To get the expression for $[H^+]$ into this form we need to convert 7.9 into a power of ten. Or, put another way, we have to find the value of x in the equation 7.9×10^x. This is easily done by consulting the table given in the "Calculation" portion of Activity X. (Note: If the exact value you are looking for is not listed in the table, use the closest value in the table.) For the example in question, we find that $7.9 \approx 10^{.9}$. By substituting this value into the equation $[H^+] = 7.9 \times 10^{-5}$ M, we obtain $[H^+] \approx 10^{.9} \times 10^{-5}$ M, or $[H^+] \approx 10^{-4.1}$ M. The pH that corresponds to $[H^+] = 7.9 \times 10^{-5}$ M is 4.1. Note that if $[H^+]$ were 8.4×10^{-5} M, we would still get the same answer. Why?

6. Determine the number of grams of ascorbic acid per liter necessary to give an initial concentration of 1×10^{-4} M. (The gram molecular weight of ascorbic acid is 176 g.)

7. Proceed to Part II and make up your test solution.

PART II: PREPARING THE TEST SOLUTION

INTRODUCTION:

As in Laboratory Activity X, the mass of ascorbic acid needed to prepare a solution at the calculated concentration is too small to be weighed out on the balances available to you. Therefore, you will once again prepare an initial solution 100 times as concentrated as the desired concentration. Then you will dilute this solution 100-fold to obtain the test solution.

MATERIALS:

ascorbic acid ($C_6H_8O_6$)	BIP
2 Erlenmeyer flasks, 125-ml	pH probe
graduated cylinder, 100-ml	stirring rod
pipet, 1-ml	spatula
balance	"prepared" water

PROCEDURE:

1. Weigh out 10 times the mass of ascorbic acid you calculated as necessary to make up your solution. (Hint: The calculated value should be in the range of

0.01 - 0.02 g per liter. If your answer is not in this range, recheck your calculations.)

2. Add to the acid enough "prepared" water to make 100 ml of solution.

3. Using the 1-ml pipet, transfer 1.0 ± 0.01 ml of the acid solution to a clean 125-ml flask and dilute to 100 ml with prepared water.

4. When there is an opportunity, proceed to the pH meter to determine the pH of your solution. Make sure that the pH probe has been carefully rinsed with distilled water before you insert it into your solution. Also remember to let the probe sit in the solution for 60 seconds before taking a reading. Record the pH of your test solution.

DISCUSSION QUESTIONS:

1. How does the pH of your solution compare with the pH obtained by calculation?

2. Discuss some of the sources of error in this activity.

ABSORBANCE-TRANSMITTANCE TABLE

Trans. (%)	Absorbance	Trans. (%)	Absorbance	Trans. (%)	Absorbance	Trans. (%)	Absorbance
0.0	-----	25.0	.600	50.0	.300	75.0	.125
0.5	2.300	25.5	.595	50.5	.295	75.5	.120
1.0	2.000	26.0	.585	51.0	.290	76.0	.120
1.5	1.825	26.5	.575	51.5	.290	76.5	.115
2.0	1.700	27.0	.570	52.0	.285	77.0	.115
2.5	1.600	27.5	.560	52.5	.280	77.5	.110
3.0	1.525	28.0	.555	53.0	.275	78.0	.110
3.5	1.460	28.5	.545	53.5	.270	78.5	.105
4.0	1.400	29.0	.540	54.0	.270	79.0	.100
4.5	1.345	29.5	.530	54.5	.265	79.5	.100
5.0	1.300	30.0	.525	55.0	.260	80.0	.095
5.5	1.260	30.5	.515	55.5	.255	80.5	.095
6.0	1.220	31.0	.510	56.0	.250	81.0	.090
6.5	1.185	31.5	.500	56.5	.250	81.5	.090
7.0	1.155	32.0	.495	57.0	.245	82.0	.085
7.5	1.125	32.5	.490	57.5	.240	82.5	.085
8.0	1.095	33.0	.480	58.0	.235	83.0	.080
8.5	1.070	33.5	.475	58.5	.230	83.5	.080
9.0	1.050	34.0	.470	59.0	.230	84.0	.075
9.5	1.020	34.5	.460	59.5	.225	84.5	.075
10.0	1.000	35.0	.455	60.0	.220	85.0	.070
10.5	.980	35.5	.450	60.5	.220	85.5	.070
11.0	.960	36.0	.445	61.0	.215	86.0	.065
11.5	.940	36.5	.440	61.5	.210	86.5	.065
12.0	.920	37.0	.430	62.0	.210	87.0	.060
12.5	.905	37.5	.425	62.5	.205	87.5	.060
13.0	.885	38.0	.420	63.0	.200	88.0	.055
13.5	.870	38.5	.415	63.5	.200	88.5	.055
14.0	.855	39.0	.410	64.0	.195	89.0	.050
14.5	.840	39.5	.405	64.5	.190	89.5	.050
15.0	.825	40.0	.400	65.0	.185	90.0	.050
15.5	.810	40.5	.395	65.5	.185	90.5	.045
16.0	.795	41.0	.390	66.0	.180	91.0	.040
16.5	.785	41.5	.380	66.5	.175	91.5	.040
17.0	.770	42.0	.375	67.0	.175	92.0	.035
17.5	.755	42.5	.370	67.5	.170	92.5	.035
18.0	.745	43.0	.365	68.0	.165	93.0	.030
18.5	.735	43.5	.360	68.5	.165	93.5	.030
19.0	.720	44.0	.355	69.0	.160	94.0	.025
19.5	.710	44.5	.350	69.5	.160	94.5	.025
20.0	.700	45.0	.345	70.0	.155	95.0	.020
20.5	.690	45.5	.340	70.5	.150	95.5	.020
21.0	.680	46.0	.335	71.0	.150	96.0	.015
21.5	.670	46.5	.330	71.5	.145	96.5	.015
22.0	.660	47.0	.330	72.0	.145	97.0	.015
22.5	.650	47.5	.325	72.5	.140	97.5	.010
23.0	.640	48.0	.320	73.0	.135	98.0	.010
23.5	.625	48.5	.315	73.5	.135	98.5	.005
24.0	.620	49.0	.310	74.0	.130	99.0	.005
24.5	.610	49.5	.305	74.5	.130	99.5	.000
						100.0	.000